MASTER OF DENTAL SCIENCE

An in vitro investigation into the effects of sports gels/drinks on human hard tissue – Consumer habits and Dentist knowledge

Keys, William F.

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An in vitro investigation into the effects of sports gels/drinks on human hard tissue – Consumer habits and Dentist knowledge

By

William F Keys

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Declaration

I, William Keys, hereby declare that I am the author of this thesis and that all references cited have been consulted by myself. I have carried out the work, of which the thesis is a record of, and it has not been previously submitted or accepted for a higher degree.

Signed Date 24/7/19

William Keys

MDSc Candidate

Matriculation Number

Certificate

I hereby certify that William Keys had fulfilled the conditions of Ordinance 39 of the University of Dundee and is qualified to submit this dissertation for the Master of Dental Science degree.

Signed [redacted] Date 24th June 2019

Prof. R. G. Chadwick

Professor and Honorary Consultant in Restorative Dentistry

Unit of Restorative Dentistry and Integrated Oral Care

Dundee Dental Hospital and School

University of Dundee

Scotland

Summary

Introduction

Tooth wear has become increasingly prevalent among all age ranges of the human population and with an increase in the numbers maintaining their dentition to later years, it can be a challenging dilemma to manage in many aspects. The increase in prevalence occurring in younger individuals can be related to dietary habits. This provides challenges in both the short and long term management of such patients within the current health care system.

Aim

To examine dietary habits in relation to sports gels/drinks/supplements of amateur athletes, the potential erosive potential of such products and to gain an insight into the opinion of general dental practitioners managing tooth wear in the practice setting. The hypothesis of this study is as follows; Sports supplements may contribute to the erosive wear of the human dentition.

Method

This study comprises of five interrelated components:

- A questionnaire (questionnaire 1) to establish dietary habits of amateur athletes
- A questionnaire (questionnaire 2) to establish clinical experience and current practice of general dental practitioners
- Determining titratable acidity of commonly used drinks/supplements using 0.1M NaOH.

Summary

- Measuring contact angle of popular drinks/supplements on 3 different surfaces (tooth/ostrich egg/glass slide)
- Measuring the effects of drinks/supplements on the surface microhardness of tooth.

Results

Fifty participants completed questionnaire one. Thirty of which were male and twenty were female amateur athletes. A range of drinks/supplements was identified for laboratory testing.

Forty two dentists replied to questionnaire two. The P value when comparing how pre 2000 graduates felt their undergraduate studies had prepared them to manage tooth wear compared to 2001 and onwards graduates was 0.54. When comparing international versus UK graduates on their undergraduate experience a P value of 0.31 was obtained. Demonstrating no statistically significant difference between year of graduation and country of qualification in attitudes towards there clinical experience of managing tooth wear.

An analysis of variance (ANOVA) of the mean titratable acidity revealed highly significant ($P<0.0001$) differences between drinks/supplements. Demonstrating some sports drinks/supplements may contribute to tooth wear.

There were significant differences ($P <0.0001$) between contact angles achieved on the different surfaces. Demonstrating there was a difference in viscosity of the various test drinks/solutions.

All but three of the test substrates demonstrated highly significant differences (P<0.01) in surface microhardness following exposure to the energy gels tested. Demonstrating some may also contribute to tooth wear.

Conclusion

The use of sports drinks/supplements is commonplace among amateur athletes and their use may be contributing to the increased prevalence of tooth wear in younger individuals participating in sport.

1. INTRODUCTION

1. Introduction

The term 'tooth wear' is a general term that can be used to describe the surface loss of dental hard tissues from causes other than dental caries, trauma or as a result of developmental disorders (Hattab *et al.* 2000). Tooth wear can be sub-classified and described by its aetiological factor i.e. erosion, abrasion, attrition or abfraction. However, in tooth wear cases the aetiology tends to be multifactorial rather than any one particular mechanism (Mehta *et al.* 2012).

Dental erosion is the irreversible loss of dental hard tissue due to a chemical process of acid dissolution but one that does not involve bacterial plaque acid, and is not directly associated with mechanical or traumatic factors, or with dental caries. **Attrition** may be defined as direct tooth-to-tooth contact wear. Where particles move across and contact the tooth surface, culminating in loss of tooth tissue, this is **abrasion** (O'Sullivan, 2013.)

Abfraction has more recently been included as a potential aetiological factor contributing to the increasing prevalence of tooth wear. This process involves occlusal stresses that by transmission produce cracks at the cervical tooth margin that predispose the surface to erosion and abrasion (Bartlett *et al.* 2006). The term abfraction means to "break away" and was coined from the work of McCoy, Lee, Eakle and Grippo in the 1980's (McCoy 1982 & Lee *et al.* 1984)

The prevalence of tooth wear in a mechanical but particularly in a chemical way is perceived globally as an increasing problem (Kreulen, 2010). In the decade between 1998 and 2008 the prevalence of tooth wear has increased from 66% to 76% (White *et al.* 2009). This increase is not uniform across the age groups; the proportion of adults with any tooth wear has increased for all age groups except those over 75 years and over. The greatest increase of 15% was noted in the age group 16-24 years (Adult Dental Health Survey 2009). The UK Adult Dental Health Survey (2009) shows a continuing decline in the percentage of people with no teeth. The proportion of adults who have lost all their natural teeth has decreased since 1978, when the first UK Adult Dental Health Survey was carried out, from 30% in 1978 to 6% in 2009 (Hellyer, 2011).

With this in mind it is not surprising that there is an increase in the prevalence of tooth wear as it is both a normal physiological process that is macroscopically irreversible and is cumulative with age (Mehta, 2012). Lambrechts *et al.* in 1989 estimated the normal vertical loss of enamel from physiological wear to be approximately 20-38 μm per annum (Lambrechts, 1989). However, this does not account for wear in the younger population and it appears dental erosion is increasing and has been linked with those who have an active lifestyle participating in sports and leisure activities (Centrewall *et al.* 1986 & Milosevic 1997). As has been demonstrated, with more of the population retaining teeth and tooth wear affecting a greater percentage at a younger age, this may create a great treatment need which is likely to be of future importance in developing dental services, incorporating prevention.

2. Literature Review

2.1 Introduction to Literature Review

A literature review was carried out and up to date as of May 2019. The literature review that follows, covers a number of key subjects related to tooth wear, specifically erosion of dental hard tissue in relation to sports nutrition and how this can be measured *in vitro.* PubMed and MEDLINE databases were searched and all literature was published in English language.

2.2 General

2.2.1 Dental Erosion

Dental erosion is a prevalent condition that occurs worldwide and has been defined as a chemical process that involves the dissolution of enamel and dentine by acids not derived from bacteria (Larsen, 1990). Erosion results in the softening of enamel and dentine, which, in combination with mechanical factors such as abrasion and attrition, results in accelerated, pathological wear of the teeth (Huysmansa, 2011) this is often described as the triad of tooth wear. In enamel, mineral is dissolved from the surface resulting in a roughened structure, should this process continue, significant and clinically visible defects could develop. The microhardness of the remaining tissue is reduced making it more susceptible to wear. The effect of acid erosion on dentine is more complex due to its structure. As with enamel, the mineral content of dentine is dissolved however, unlike enamel the organic portion remains. Although erosive lesions can be created easily using an experimental model, further investigation is required into the histological features of erosive wear *in vivo* to explore how meaningful these experimentally created lesions are when developing preventative, non-invasive and restorative treatment strategies is needed (Ganss, 2014). These *in-vitro* studies have allowed for further development of management strategies for tooth wear lesions and in 2017 a decision flow chart (fig 2.1) was developed at a European Consensus meeting aiming to help guide clinicians in appropriate management choices (Loosman, 2017).

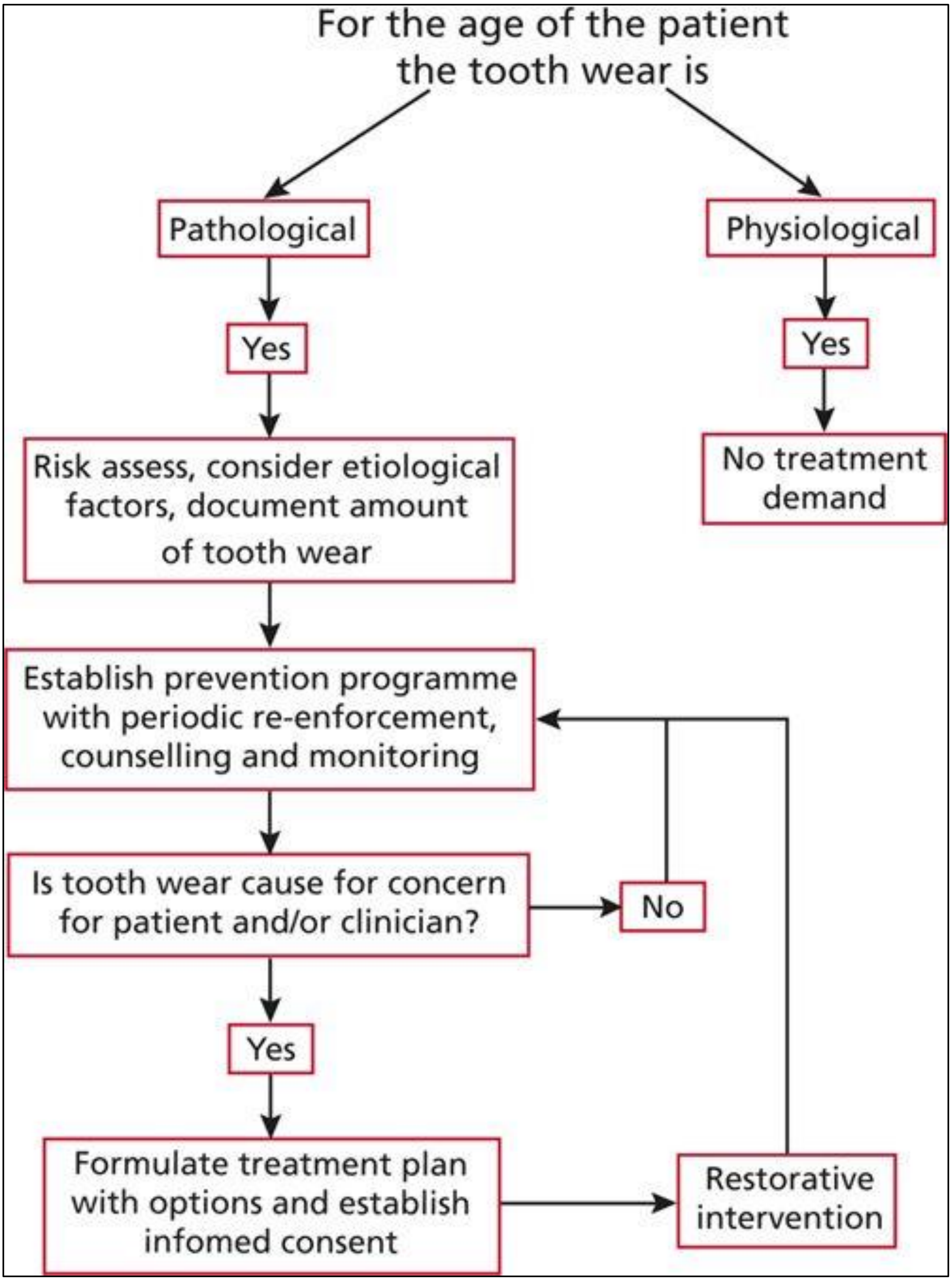

Figure 2.1. European Consensus on tooth wear management flowchart

2.2.2 Classification

There have been many attempts to construct a tooth wear index as there are both clinical and scientific needs to record tooth wear. Broadly speaking, these indices can be divided into either quantitative or qualitative, with quantitative relying on physical measurements and qualitative on the physical description and interpretation of the clinical scenario.

The most widely known tooth wear indices are those described by Eccles (Eccles, 1979) (Table 2.1). This concept was further developed and expanded upon by Smith & Knight (Smith, Knight 1984) in the 1980’s. Such tooth wear indices have been developed primarily for research and epidemiology, but they have also been used to grade and monitor wear clinically (Bardsley, 2008). However as there as yet has been no universally agreed index measuring tooth wear comparing studies is difficult and care must be taken to ensure a common index is used when monitoring wear for an individual patient.

Eccles originally classified lesions broadly as early, small and advanced, with no strict criteria definitions, thus allowing wide interpretation (Bardsley, 2008).

Grade	Clinical Appearance
I	Superficial lesions involving enamel only.
II	Localised lesions involving dentine for less than ½ of the surface.
III	Generalised lesions involving dentine for more than 1/3 of the surface
(a)	Facial surfaces
(b)	Lingual and palatal surfaces
(c)	Incisal and occlusal surfaces
(d)	Severe multisurface involvement

Table 2.1 Eccles tooth wear classification (modified by Bardsley 2008)

Smith and Knight took Eccles' ideas a stage further, producing the tooth wear index (TWI, Table 2.2), a comprehensive system whereby all four visible surfaces (buccal, cervical, lingual and occlusal/incisal) of all teeth present are scored for wear, irrespective of how it occurred (Bardsley, 2008). The lesion is scored between 0 and 4 depending on the level of wear however, clinically its use is limited as it does not describe the aetiology of tooth wear but only its severity, however, it was a modified version of this index which was used in the most recent UK Adult Dental Health Survey in 2009.

Score	Surface	Criteria
Tooth wear index according to Smith and Knight		
0	B/L/O/I	No loss of enamel surface characteristics
	C	No loss of contour
1	B/L/O/I	Loss of enamel surface characteristics
	C	Minimal loss of contour
2	B/L/O	Loss of enamel exposing dentine for less than one-third of the surface
	I	Loss of enamel just exposing dentine
	C	Defect less than 1mm deep
3	B/L/O	Loss of enamel exposing dentine for more than one-third of the surface
	I	Loss of enamel and substantial loss of dentine
	C	Defect less than 1-2mm deep
4	B/L/O	Complete loss of enamel, or pulp exposure, or exposure of secondary dentine
	I	Pulp exposure or exposure of secondary dentine
	C	Defect more than 2mm deep, or pulp exposure, or exposure of secondary dentine

Table 2.2 Smith and Knight Tooth Wear Index.

In 2008, Bartlett *et al*. introduced the Basic Erosive Wear Examination (BEWE), designed to provide a simple scoring system that can be used with the diagnostic criteria of all existing indices aiming to transfer their results into one unit, which is the BEWE score sum. The hope was that this would become a commonly adopted, reliable and reproducible scoring method, which would aid in the diagnosis and management of tooth wear (Bartlett, 2008). Similar to the Basic Periodontal Examination (BPE) in concept the BEWE divides the dentition into sextants, all surfaces are examined and the worst affected surface in each individual sextant is allocated a score ranging from 0-3 (Table 2.3).

Table 1
Criteria for grading erosive wear

Score	Criteria
0	No erosive tooth wear
1	Initial loss of surface texture
2[a]	Distinct defect, hard tissue loss <50% of the surface area
3[a]	Hard tissue loss $\geq$50% of the surface area

Table 2.3. BEWE sextant scores

Once each sextant has been scored, these values are added together and a level of erosive risk and suggested management is established from the table below (Table 2.4). As yet this screening tool has not found the popularity the BPE has. It is also difficult to find reliable evidence reporting the reproducibility of BEWE and BPE scoring in clinical practice.

Complexity levels as a guide to clinical management

Susceptibility Level	Cumulative Score of All Sextants	Management
	≤2	Routine maintenance and observation Repeat at 3-year intervals
Low	3–8	Oral hygiene and dietary assessment and advice. Identify the main causative factor(s) and develop strategies to eliminate their effects, routine maintenance, and observation. Repeat at 1- to 2-year intervals
Medium	9–13	As above plus Measures to increase the resistance of tooth surfaces Ideally, avoid the placement of restorations and monitor erosive wear with study casts, photographs, or silicone impressions Repeat at 6- to 12-month intervals
High	≥14	As above plus Especially in cases of severe progression consider special care, which may involve restorations Repeat at 6-month intervals

Table 2.4. Cumulative BEWE scores from all sextants.

It was hoped that use of the BEWE system would become the international standard and thereby contribute toward more optimal understanding, evidence based treatment, and prevention of tooth wear (Vered 2014).

2.2.3 Prevalence of tooth wear

The prevalence of a disease is the proportion of a population that are cases at a point in time (Chapter 2. Quantifying disease in populations, BMJ). It is calculated using the formula below:

Prevalence = Number of existing cases on a specific date ÷ Number of people in the population on this date.

It is commonly used in medical literature for chronic conditions as opposed to incidence, which is the rate at which new cases occur in a population during a specified period (Chapter 2. Quantifying disease in populations, BMJ).

As prevalence provides us with a snap shot of a chronic condition at a particular time it is appropriate to use when reporting on tooth wear.

Despite tooth wear being a common condition, little data exists concerning prevalence. Of the studies available, most are concerned with children and adolescents and only a small number refer to forms of tooth wear in adults. (Ganss *et al*, 2011). This may possibly be due to the easier accessibility of younger generations to participate in surveys whilst in the educational system. (Schlueter *et al,* 2018).

As expressed by Schlueter *et al.* (2018) tooth wear is a common condition in the general population and whilst it has become increasingly prevalent, a discussion around the prevalence of dental erosion is not as simple as it may seem.

When comparing studies of prevalence, there is great variation on the reported prevalence of tooth wear ranging from 100% (Jaeggi, 2004) – 4% (Lussi *et al.*1991). The number of studies addressing the prevalence of dental erosion has increased over the years (fig. 2.2) A PubMed search revealed a total of 931 publications using the terms ‘tooth wear OR dental erosion AND prevalence.’ These studies are difficult to compare due to the variations in the studies such as; study sample (age, risk groups and dentition), standards set (calibration, indices used and procedures) and choice of outcomes studied (Schlueter *et al* 2018).

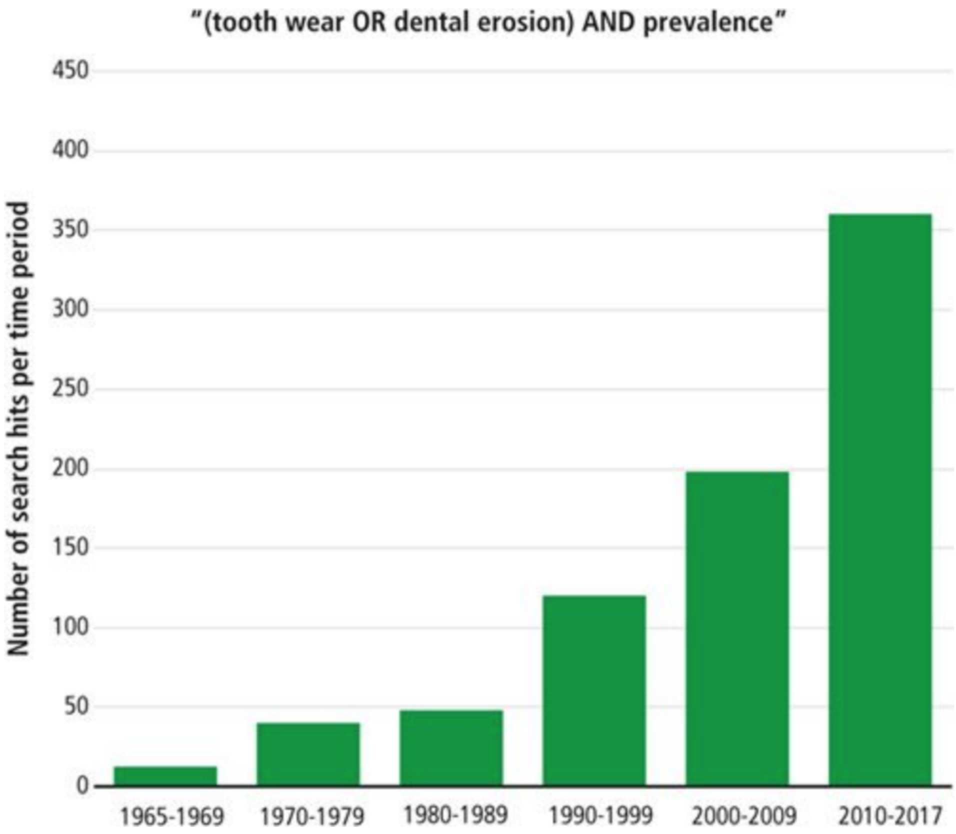

Figure 2.2 The increase in published tooth wear prevalence studies (Schlueter *et al.* 2018).

The UK Children's Dental Health Survey 2013, reported a third of five year olds in the UK had evidence of tooth wear on one or more of the buccal surfaces of the primary upper incisors, whilst four percent had tooth wear involving dentine or the pulp. Tooth wear of the lingual surfaces was more common, with 57% of five year olds being affected. Wear progressing to dentine or pulp was present on 16% of lingual surfaces (Table 2.5). (Children's Dental Health Survey, 2013)

England, Wales and Northern Ireland, 2013		Percentages
Children aged 5	Any TSL	Into dentine or pulp
Buccal surfaces		
England	35	4
Wales	11	3
Northern Ireland	24	7
Total	33	4
Lingual surfaces		
England	57	15
Wales	47	17
Northern Ireland	67	24
Total	57	16

Table 2.5 Percentage of five year old children with tooth surface loss on the surfaces of primary incisors

The same survey went on to assess permanent teeth in children and adolescents demonstrating that, a quarter of 12 year olds were reported to have tooth wear on their first permanent molar teeth and the buccal surface of their maxillary incisors (25% and 24%). As with primary incisors, tooth wear on permanent incisors was more common on the lingual surfaces than on the buccal surfaces, affecting 38% of children.

The proportion of children with any tooth wear at age 15 on the occlusal surface of the molar was higher than at age 12 (31% compared to 25% Table 2.6).

England, Wales and Northern Ireland, 2013 — Percentages

Children aged 12, 15	England	Wales	Northern Ireland	Total
Incisors				
Buccal surfaces				
12 year olds	26	5	21	24
15 year olds	27	11	29	27
Lingual surfaces				
12 year olds	39	30	44	38
15 year olds	43	40	57	44
Molars				
12 year olds	26	12	12	25
15 year olds	32	17	18	31

Table 2.6. Percentage of children aged 12 and 15 with any tooth surface loss on the surfaces of permanent incisors and first permanent molars.

In clinical practice, it is important to identify those children who suffer from tooth wear at a young age, as tooth wear is a cumulative process, so appropriate preventative measures may be instigated. It has been demonstrated that as a person ages tooth surface loss is likely to continue and may progress from a mild chronic condition to one that is severe. (Harding *et al.* 2010). It is well accepted that the mechanical resistance of deciduous teeth is lower than that of permanent teeth. However, there is no consensus on whether deciduous teeth are more susceptible to erosion (Hunter *et al* 2000.)

With regard to the adult dentition, Van't Spijker and colleagues carried out a systematic review of the prevalence of tooth wear in the adult population. They concluded that the prevalence of adults presenting with severe tooth wear increases from 3% at the age of 20 years to 17% at age of 70 years. Thus, increasing levels of tooth wear was significantly associated with age. (Van't Spijker et al. 2009)

The UK Adult Dental Health survey 2009 demonstrated that the prevalence of tooth wear extending into dentine was high with over three quarters (77%) of dentate adults showing some tooth wear in their anterior teeth, of which 15% was moderate wear and 2% severe wear. It was also noted that there had been an upward trend in the prevalence of tooth wear since the previous adult dental health survey conducted 10 years earlier and, there was an increase in moderate tooth wear amongst younger individuals. “Moderate tooth wear in

16 to 34 year olds is of clinical relevance as it is suggestive of rapid tooth wear." (Adult Dental Health Survey 2009).

On a global scale, again there are large ranges of prevalence in tooth wear published in the literature. For example; Australia 0-33% (Huang *et al.* 2015), Greece 52-79% (Mantonanaki *et al.* 2013), India 29% (Nayak *et al.* 2010), Ireland 47% (Harding *et al.* 2003) and Switzerland 100% (Jaeggi 2004). These figures further demonstrate the difficulties identifying the true prevalence of tooth wear globally.

2.2.4 Diagnosis of tooth wear

The diagnosis of tooth wear can be difficult especially when it is minimal and confined to enamel as it can be hard to identify with the naked eye. Clinical appearance is the most important feature for dental professionals to base their diagnosis upon. (Lussi & Jaeggi, 2008) Often, tooth wear, that is primarily erosive in nature, tends to leave the enamel with a smooth silky-glazed appearance. (Lussi *et al*. 2008) When wear has extended into dentine it is much easier to diagnose due to changes in colour. A diagnosis of dental erosion is made more difficult due to the previously discussed triad of tooth wear mechanisms and therefore careful history taking is important. (O'Sullivan & Milosevic, 2013.)

In 2015 in recognition of the difficulties in the diagnosis of erosion, the European Federation of Conservative Dentistry constructed a consensus report on the diagnosis and management of dental erosion. As well as recommending the BEWE as a suitable screening and classification tool, it also highlighted the importance of appropriate medical and dental history taking. (Carvalho *et al.* 2015) As part of this information gathering diet analysis should be carried out, (Monihyan 2002) this involves use of a diet diary which should be carried out over a three day period and include at least one day of the weekend.

2.2.5 Aetiology

Erosion cannot occur without the presence of acid. Major destruction of tooth mineral content typically occurs below a critical pH of 5.5. In the case of dental erosion, acid can be from an intrinsic or extrinsic source (Lussi and Jaeggia 2004) either singly or in combination. Extrinsic acids are those, which enter the oral cavity through ingestion or an individual maybe exposed to an acid environment. Intrinsic acids are defined as those acid which enter the oral cavity from the stomach i.e. vomiting.

2.3 Extrinsic acids and dental erosion

2.3.1 Drinks

In 2015, 13.3 billion litres of soft drinks were consumed in the UK with the average intake of 203.61 litres per capita as reported by the British Soft Drinks association.

There is significant evidence which demonstrates that carbonated drinks have erosive potential. Moazzez *et al*. (2000) reported drinking carbonated drinks and the method and speed of drinking influenced dental erosion. They concluded that, health promotion may need to be directed towards modifying drinking habits as well as the frequency of intake of acidic drinks to prevent erosion. (Moazzez *et al.* 2000). 42% of fruit drinks are consumed by children aged 2-9 years old (Rugg-Gunn *et al* 1987). *In-vitro* investigation of these fruit drinks and smoothies indicate they have a high titratable acidity (Blacker & Chadwick 2013). They produced a significant erosion of enamel in vitro (Tahmassebi *et al.* 2014). Interestingly, the consumtion of fruit smoothies has been recommended in the five a day campaign to promote a healthy diet (NHS Choices. 5 a day: what counts? 2009).

Alcoholic drinks including wine, ciders and alco pops (Rees *et al.* 2002) not only can be a source of extrinsic acid themselves but also of intrinsic acid for alcohol has been shown to increase the occurrence of gastric reflux, a source of intrinsic acid. (Robb *et al*.1989).

2.3.2 Food

There is high public awareness of diet as a causative factor for dental caries. However there is less awareness of the destruction of the dentition by non-carious tooth surface loss and the impact diet may have on this. Healthy diets, such as fruits and fruit juices may cause erosion by their acidity. Furthermore, acidity can be an essential element in certain sour dishes (Wongkhantee *et al*. 2006). Milosevic et al. showed that crisps, ketchup, brown sauce and vinaigrettes all have erosive potential (Milosevic *et al*. 2004) whilst Caglar *et al*. showed yogurt had no erosive potential as the acidic effects were nullified by the presence of calcium with the diary component (Caglar *et al*. 2006). This is despite the fact yogurt can have a pH less than 4, however due to the high mineral content maintains the supersaturation of minerals with respect to tooth structure (Lussi *et al.* 2012.)

2.3.3 Environmental

Environmental acid exposure has also been associated with dental erosion and is frequently documented in case reports (Wiegand *et al*. 2007). It has been demonstrated that those who work in an acidic atmosphere such as battery manufacturers are at an increased risk of dental erosion (Johansson *et al*. 2005). Others who have been shown to have an increased risk are professional wine tasters and those swimmers using poorly monitored chlorinated swimming pools (Centerwall *et al.* 1986). A systematic review on the oral health of athletes reported prevalence values of tooth wear to be between 36 and 86%. (Ashley *et al*. 2015).

2.4 Intrinsic acid and dental erosion

2.4.1 Medically relevant factors

Intrinsic acid, which causes dental erosion, is gastric in origin. There are a number of medical conditions, which have been associated with dental erosion (listed below).

- Vomiting/Regurgitation
- Rumination
- GORD (gastro-oesophageal reflux disease)
- Xerostomia

Any condition associated with gastric acid reaching the oral cavity, may cause erosion of the dental hard tissues (Valena *et al*. 2002) as the stomach contains hydrochloric acid, which has a pH of 1. However, the clinical manifestations often take many years before becoming evident to a patient and only after cumulatively repeated exposure to gastric contents (Scheutzel, 1996).

Uhlen *et al.* identified that erosion commonly affects individuals with Bulimia Nervosa (a self-induced vomiting disorder), and is more often found on the palatal and lingual surfaces of teeth as these are in the path of the vomit (Uhlen *et al.* 2014).

Rumination is the voluntary movement of the stomach contents into the mouth, this is then held in the mouth leading to erosion of the palatal and buccal surfaces (Gilmour *et al.* 1993). Little is known about rumination and its prevalence. Diagnosis can be difficult as many patients are either unaware of such a habit or are unwilling to disclose it when a dental history is taken. GORD (gastro-oesophageal reflux disease) is a very common condition, with a prevalence of up to 10–20% in the general population (Dent *et al.* 2005). Holbrook et al. and Bartlett et al. demonstrated by oesophageal pH monitoring, that patients with dental erosion commonly suffered from GORD (Holbrook *et al*. 2009; Bartlett *at al.* 1996)

Xerostomia is the reduction of saliva in the mouth and this can contribute to dental erosion. Although xerostomia itself does not cause wear of the dental hard tissues, saliva acts as a buffer to acids, which are present in the oral cavity and without this natural buffer dental erosion can become more severe and occur over a shorter period of time. Oral dryness can be recorded using the Challacombe Scale (figure 2.3 Jawad *et al*. 2015) and such a tool may have potential in the diagnosis of tooth wear.

Table 1	Clinical oral dryness score
1	Mirror sticks to buccal mucosa
2	Mirror sticks to tongue
3	Saliva frothy
4	No saliva pooling in the floor of the mouth
5	Tongue shows generalised shortened papillae
6	Altered gingival architecture (ie smooth)
7	Glassy appearance of oral mucosa especially palate
8	Tongue lobulated/fissured
9	Cervical cavitations on more than two teeth
10	Debris on palate or sticking to teeth

Figure 2.3. Challacombe Scale.

2.5 Modifying factors in the development of dental erosion

2.5.1 Role of Saliva

Three major salivary glands; the parotid, submandibular and sublingual as well as numerous minor salivary glands secrete between 1 and 1.5 litres of saliva per day in the average adult (Cole *et al.* 1988). Saliva contains both organic and inorganic components. Among the inorganic components, bicarbonate is related to saliva buffering capacity, while calcium and phosphate allow for maintenance of tooth mineral integrity (Buzalaf *et al.* 2012). Among the organic components, there are a number of proteins and glycoproteins that may influence several aspects of oral health (Dodds *et al.* 2005)

Glycoproteins adhere to the enamel surface of teeth forming a salivary pellicle which can act as a barrier to acid attack of the dental hard tissues. A reduction in saliva will reduce the presence of not only the inorganic buffering constituents of saliva but also the formation of this protective barrier. A number of studies have shown that erosion may be associated with low salivary flow or/and low buffering capacity (Lussi *et al.* 2011).

2.5.2 Enamel

Enamel is a highly mineralised (approximately 96% by weight) tissue consisting of myriads of uniformly wide, fairly well orientated crystals of hydroxyapatite tightly packed into an organic matrix (Boyde 1967) and is the hardest tissue in the human body. When fluoride, from the diet or oral hygiene measures combines with hydroxyapatite a more stable compound of fluorapatite is formed which is more resistant to acid dissolution (Featherstone 2000.) due to a lower critical pH of 4.5.

2.5.3 Dentine

Dentine forms part of the pulpo-dentinal complex. Dentine is formed by odontoblasts and broadly speaking there are three types of dentine; Primary dentine formed during tooth development, secondary dentine formed slowly throughout the life span of the tooth and finally tertiary dentine, laid down as a repair/defence mechanism due to dental caries or wear (Orchardson and Cadden. 2001). Dentine is much less mineralised than enamel (approximately 70% by weight) and has a tubular structure formed of hydroxyapatite supported by a network of collagen. Thus, dentine is more susceptible to dental erosion than enamel.

2.5.4 Bruxism

Bruxism has been defined as non-functional (parafunctional) movements of the mandible, with or without audible sound occurring during the day or night (Khan *et al.* 1998). It is commonly managed by advice and provision of a splint (Hemmings *et al.* 2018). Bruxism in combination with the repeated acid attack by either intrinsic or extrinsic origin can result in significant erosion defects, as the acid challenge will soften enamel and dentine surfaces making the tooth surface more prone to degrade with the grinding of tooth surfaces against each other.

2.6 Sport as a modifying factor in dental erosion

2.6.1 Sport and salivary flow rate

During sport, the body's temperature is raised; this increase in temperature can vary depending on the intensity and duration of exercise as well as the heat of the environment in which the exercise occurs. As the body temperature increases water is lost in the form of respiration and sweat. It is extremely important to ensure fluids are taken during exercise to ensure the fluids excreted by the body are replaced, should this not be achieved a state of dehydration may occur.

Hydration is extremely important for the oral cavity as dehydration is associated with decreased parotid salivary gland flow rates (Ship and Fischer 1997). Ford et al. found that dehydration and exercise without regular fluid intake can reduce the salivary flow rate (Ford *et al. 1997*), so those participating in all levels of sport should be advised to maintain hydration and stimulate saliva flow by drinking water regularly during exercise (Walsh 1999). This decreased salivary flow may increase susceptibility to dental erosion.

2.6.2 Sports nutrition

Nutrition is a key factor influencing health (Dixey *et al.* 1999). Food is a source of energy and as more energy is expelled during exercise nutrition must be altered to balance for this increase in expenditure. Many athletes have utilized diet as a component of their training program, and dietary intervention has had a positive effect on performance (Grandjean, 1997).

As part of this diet, supplements are commonly used such as energy gels, protein shakes and sports drinks. “Nutritional intake, including usual diet, sports drinks and supplements is a major determinant of oral health, including dental caries, periodontal disease and dental erosion” (Needleman *et al.* 2014). There has been much evidence over the years on the effects of sports drinks on the dentition however, little is known about the effects of many other commonly used sports supplements and their erosive potential.

2.6.3 Sports drinks and erosion

Sports drinks are beverages that are specially formulated to help people rehydrate during or after exercise (Diabetes UK website). These are extremely popular with worldwide demand; the U.S. market alone is estimated at being worth over $1.5 billion a year (Coombes, 2005). There is significant evidence available that consumption of such beverages at frequent intervals may lead to dental erosion. Milosevic examined the pH of 8 common sports drinks and demonstrated that all of these beverages had a pH of less than 5.5 and would therefore cause dental erosion (Milosevic, 1997). It is important when recording a dental history that consumption of these drinks is recorded.

2.6.4 Protein shakes

Protein supplements contain high levels of protein and can come in various forms i.e. shakes, powders, bars and gels. “These supplements are frequently consumed by athletes and recreationally active adults to achieve greater gains in muscle mass and strength and improve physical performance" (Pasiakos *et al.* 2015). It was reported that the sale of sports nutrition products and in particular whey protein has doubled between 2007 and 2012 with £260 million being spent on these products in the UK alone. It's now estimated that it will be worth around £8 billion by 2017. One in ten men use such products at least once a week. (The Economist & The Independent 2015 Websites).

Despite their common use amongst those participating at sport on every level, little is known about the effect of protein supplements on the human dentition.

2.6.5 Energy gels

Energy gels were first introduced in the 1980's to effectively deliver an easily digestible and quick supply of carbohydrate for energy during exercise. They have often been described as a mix between energy bars and sports drinks. Commercially available energy gels are essentially sachets of complex carbohydrates such as Maltodextrin, Fructose and electrolytes. Once consumed, the carbohydrates found in the gels are absorbed into the blood to supply the body with calories and nutrients to fuel exercise activity by helping to delay muscular fatigue, raise blood sugar levels, and enhance performance. It was found that amongst a group of elite long distance swimmers, energy gels along with bananas and honey were the most common forms of nutritional intake during a race (Papadopoulou *et al.* 2011). Again however, as with many other sports supplements, little is known about the potential effects of energy gels on the human dentition.

2.6.6 Oral health and sports

Poor oral health amongst elite athletes has commonly been reported. There are many potential challenges to athlete's oral health, including nutrition, oral dehydration, exercise-induced immune suppression, lack of awareness, negative health behaviours and lack of prioritisation (Needleman *et al.* 2014). This was highlighted during the 2012 London Olympic games when 30% of all visits to the medical clinic were dentally related. This was the second highest cause of medical attendance at these games; only musculoskeletal problems had a higher rate of medical visits. (Vanhegan *et al*. 2013).

2.7 Measuring dental erosion potential

Over the years there have been many studies, which have investigated the erosive potential of various drinks (Coombes 2005, Rees *et al.* 2006). These studies have focused on measuring the acidity of these drinks as well as the effects these drinks have had upon the dental hard tissue surface. In order to understand the results of such studies, an understanding of acid base chemistry is required.

2.7.1 Acid base chemistry

The acidity of a solution is measured as pH, this is a measure of the initial hydrogen concentration and is measured using a logarithmic scale ranging from 0-14. A pH of seven is neutral, below pH 7 is acidic and greater than pH 7 is alkali. Mathematically pH is defined as;

$$\mathbf{pH = -\log_{10}[H^+]}$$

As an example a solution with a pH of 1 is a strong acid whilst a pH of 13 is a strong base. When an acid is added to water hydrogen ions are produced however, when a base is added to water hydroxide ions are formed. A strong acid is one which fully dissociates in water whilst a weak one only partially dissociates (Masterton *et al.* 1989). It should be noted that a shift in one pH unit represents a ten fold alteration in the hydrogen ion concentration.

There are a variety of methods available to test pH ranging from the simple pH indicator paper to titration of an acid solution with a base; commonly sodium hydroxide (NaOH) is used. The amount of 0.1M sodium hydroxide in mls required to raise the pH of a solution to 7 is called the titratable acidity and it is this method, which give a more accurate indication of the erosive potential of a drink (Shaw and Smith 1999). Syed and Chadwick have suggested that in order to make inter study comparison more readily achievable the standardized titratable acidity (STA) should be used (Syed & Chadwick, 2009). This may be calculated with the following formula;

STA= (mean volume in ml of 0.1M sodium hydroxide required to neutralise 25ml of solution x 0.0001) x 40

STA is defined as the mean volume of 0.1M sodium hydroxide required to neutralize one litre of solution/gel.

2.7.2 Measurement of tooth surface loss

There are a number of ways in which tooth surface loss can be measured when studied and these vary for *in-vitro* and *in-vivo* studies.

The measuring of tooth surface loss *in-vitro* can be challenging and we must bear in mind that all of these studies have been carried out in a laboratory and as such, how these results may relate to clinical situations must be taken into account in interpretation of the results. Although many variables can be controlled such as exposure times and concentrations, the conditions of the oral cavity cannot be replicated and *in-vitro* studies must only be used to inform and guide clinical practise.

In-vitro measuring techniques used have their own advantages and disadvantages;

- Macroscopic examination is easy and quick to carry out but displays poor sensitivity.
- Surface profilometry measures the actual depth of erosion but in a 2D field.
- Microhardness compares the hardness before and after exposure of a solution but may not measure the full extent of hard tissue loss.
- Calcium and phosphate dissolution is reproducible but comparison in human teeth can be difficult.
- Digital image analysis is expensive but very precise.
- Scanning electron microscopy produces a high resolution and depth of field but shrinkage of tooth specimens during preparation occurs.

2.7.3 Surface profilometry

A surface profilometer is an instrument, which can determine the amount of tooth surface that has been lost by scanning the surface. The tooth surface is scanned prior to exposure to the solution, this involves a diamond stylus contacting the tooth surface and crossing it in different directions. Once the specimen has been exposed to the solution, the scan is repeated and the amount of tooth surface loss is calculated by a software package, which expresses the results in μm. This technique has been commonly used to assess the erosive potential of many drinks *in-vitro* (Rees *et al.* 1998). However, this method over-estimates tooth loss up to a factor of 10 and tooth wear can only be measured in two dimensions (Addy *et al.* 2000).

2.7.4 Tooth microhardness

The hardness of a material is an indication of its resistance to penetration when indented by a hard asperity (McCabe *et al.* 1998). There are a number of instruments, which can be used to examine the hardness of a material. The instrument, which I intend to use in this project, is the Vickers hardness tester. This consists of a diamond pyramid indenter. Once the indent is made, the specimen is examined under a microscope and a hardness number is calculated based on the distance across the diagonal axes of the indent. The results are recorded as Vickers hardness numbers (VHN) and the higher the number the less the resistance to penetration and therefore, the harder the surface is.

Vickers hardness numbers of some relevant dental materials have been summarised (Table 2.7) (McCabe, 1998).

Material	VHN
Enamel	350
Dentine	60
Dental amalgam	100
Co/Cr alloys	420

Table 2.7 Vickers hardness numbers of some commonly used dental materials.

In testing the erosive potential of a solution, the Vickers hardness tester is used prior to and post exposure of the tooth substrate to the test solution and the difference in VHN is recorded. To adjust for specimen variation, the percentage change in hardness is reported. This will give an indication of how the solution has affected the surface of the test tooth.

2.8 Contact angle

Contact angle is a quantitative measure of the wetting of a solid by a liquid. Changes in the solid or the liquid can affect the resultant contact angle; wettability is the ability of a solid surface to reduce the surface tension of a liquid in contact with it such that it spreads over the surface and wets it, this may have an impact on how various solutions contribute to non-carious tooth surface loss.

Contact angle is defined geometrically as the angle formed by a liquid at the three-phase boundary where a liquid, gas and solid intersect. The Young equation (below) describes the balance at the three-phase contact of solid-liquid and gas. In this equation, γ_{sv} represents, surface tension forces of a liquids vapour, γ_{sl} represents, surface tension forces of solid substrate, γ_{lv} represents surface tension forces of liquid.

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos \theta_Y$$

Such a force vector approach is vital to understanding the spread of droplet (figure 2.4). It illustrates how changing the surface substrate will alter the contact angle (Darvell, 2018).

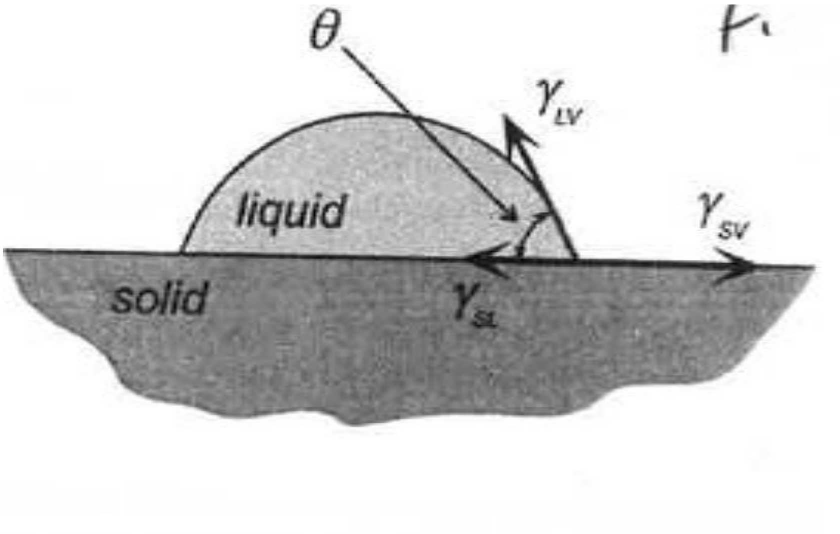

Figure 2.4. The force vector which may be considered to act at the contact line of a drop on a solid surface (Darvell 2018).

From the figure below (Fig. 2.5), it can be seen that the low contact angle values indicate that the liquid spreads on the surface while high contact angle values show poor spreading. If the contact angle is less than 90° it is said that the liquid wets the surface, zero contact angle representing complete wetting. If contact angle is greater than 90°, the surface is said to be non-wetting with that liquid.

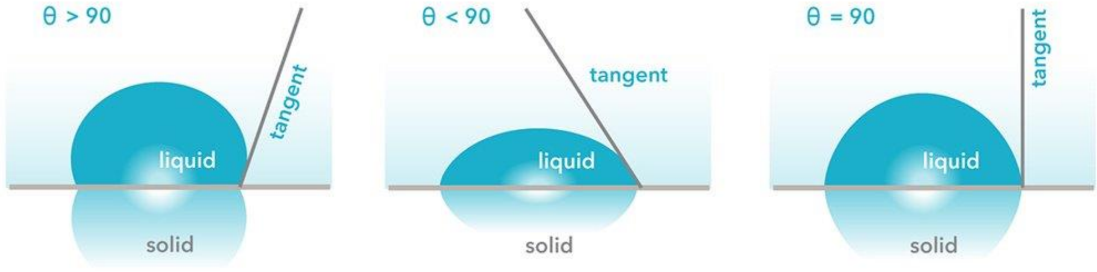

Figure 2.5 Contact angles of these liquids in contact with a solid indicating various wetting properties of liquid.

There are a number of ways to measure contact angle, one such technique is to place a droplet on the solid surface and an image of the drop is recorded. The static contact angle is then defined by fitting Young equation around the droplet.

The smaller the contact angle is, the greater will be the effect a liquid may have upon the surface topography. This is because for a low contact angle a greater surface area of substrate is covered by the droplet of liquid than when a large contact angle is observed.

2.9 Calcium and phosphate dissolution

Another method for determining tooth surface loss *in vitro*, has been photometric determination of calcium and phosphate release. Arsenazo III a calcium dye reagent reacts with calcium in an acid solution forming a blue purple complex. The colour intensity developed is proportional to the calcium concentration. Malachite green reacts with phosphate to a coloured complex, which can also be determined photometrically (Hannig *et al.* 2005). Thus by determining the photometric properties of solutions of known concentrations of these ions, calibration curves may be constructed against which the photometric values of unknown solutions may be compared to determine ion concentration and therefore tooth surface loss.

2.10 Digital terrain mapping

As we have established, tooth wear is increasingly prevalent and monitoring changes to an individual's dentition over time can be very difficult as these changes often happen over a prolonger period of time. Techniques to monitor such wear must be significantly sensitive to detect small amounts of wear that, in themselves, may be of little significance but cumulatively may result in significant loss of tooth surface (Chadwick *et al.* 1997.) Comparing study models over time is not a sensitive and reproducible technique and so models can now be digitised and compared accurately and in a reproducible fashion to allow for accurate monitoring of tooth wear and its rate of progression.

2.11 Scanning electron microscopy

A scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology and crystalline structure allowing SEM to be a very useful tool in the measurement of tooth wears. This technique has been used *in vitro* studies assessing the effects of various solutions/acids on enamel (Maas, 1994).

2.12 Conclusion

As I have identified from this literature review there has been extensive research carried out on tooth surface loss and the role many dietary substances can have. I have also identified that athletes appear to be at high risk for dental disease and although there is evidence on the erosive potential of sports drinks, there would appear to be no research on the effect of sports nutritional supplements such as protein shakes and energy gels on dental hard tissue despite their growth in popularity. I hope that the work produced in this thesis will inform both professionals and those participating in sports whether these supplements cause dental erosion or not.

The hypothesis of this study is as follows; Sports supplements may contribute to the erosive wear of the human dentition.

3. MATERIALS AND METHODS

3. Materials and methods

3.1 Introduction

This study consisted of five interrelated elements:

1. **Establishing the habits** - of those participating in amateur level sports who consumed sports drinks and nutritional supplements.
2. **Establishing experience and current practice** - of local general dental practitioners in managing toothwear.
3. **Determining the titratable acidity** - of ten varieties of shop bought sports drinks and supplements.
4. **Measuring the contact angle** - of the most popular drinks/supplements identified by the survey on three different surfaces (glass side, tooth specimen and ostrich egg specimen).
5. **Measuring microhardness changes on tooth structure** – of five popular flavours of SIS sports gels.

1. A questionnaire (questionnaire 1) was designed and administered to identify trends and levels of consumption of sports drinks and nutritional supplements. Once the questionnaire was constructed, the questionnaire was administered to a group of 10 participants to ensure it was easily understood and appropriate data could be extracted. Once, this test sample was completed, the questionnaire was completed by 50 consenting individuals who competed in a range of amateur sports.

2. A questionnaire (questionnaire 2) was designed and administered to identify current practices and confidence levels in managing tooth wear of local general dental practitioners. The questionnaire was circulated to local general practitioners and three reminder emails were sent. A total of 43 questionnaires were returned completed.

3. The ten varieties of supplement identified in questionnaire 1 were tested for their initial pH and titratable acidity

4. The contact angle of ten popular solutions/gels (identified by the questionnaires) was then measured. This allowed the wettability of each solution on various surfaces to be established.

5. Five different flavours of one particular brand of sports gel were exposed to human dental hard tissue and microhardness was measured pre and post exposure.

All of these tests were also performed for negative (Volvic™ still mineral water) and positive (Tropicana™ smooth orange juice) control drinks with the exception of microhardness testing.

As the investigation involved the use of extracted human teeth and the administration of questionnaires. The advice of the East of Scotland Research Ethics Service, Tayside medical science Centre was sought. They advised that as teeth to be used in in the experiments were collected prior to September 2006 ethical approval was not required.

3.2 Participant Questionnaire

Using the Dillman principles (Dillman, 1941) for Questionnaire construction, a simple one page questionnaire was designed and piloted upon a convenience sample, this was then re-run on the same convenience sample a number of weeks later to assess readability and reproducibility of the questionnaire. Upon completion of this, the final questionnaire was distributed at sporting events and to local sporting clubs. Completed questionnaires were collected by the author and taken to Dundee Dental Hospital and School for data input and analysis. The administered questionnaire is contained in Appendix A.

A relational database using Paradox (Paradox, Version 3.5, Borland International, CA 95067-0001. USA) was created for collation of the data from the completed questionnaires and its subsequent interrogation. Statistical analyses were performed using statistical programmes within Statistics for Social Scientist (Cohen & Holliday, 1982) (chi-square) and Excel (Microsoft® Office 2010, Version 14.6.4) (sample descriptive statistics). The threshold value used for statistical significance was conventional that is 0.05 ($P < 0.05$).

3.3 Dentist Questionnaire

A second simple one page questionnaire using the same principles as the one for participants was constructed and distributed to local general dental practitioners via the practitioners services of NHS Grampian. The NHS Grampian practitioners service were contacted via email and asked if they would be willing to circulate the questionnaire to dentist who were registered to treat NHS patients with the trust. Once the ethical approval was granted, they agreed to circulate the questionnaire (appendix B). Completed questionnaires were collected by the author and taken to Dundee Dental Hospital and School for data input and analysis. The administered questionnaire is contained in Appendix B.

A relational database using Paradox (Paradox, Version 3.5, Borland International, CA 95067-0001. USA) was created for collation of the data from the completed questionnaires and its subsequent interrogation. Statistical analyses were performed using statistical programmes within Statistics for Social Scientist (chi-square) and Excel (sample descriptive statistics). The threshold value used for statistical significance was conventional that is 0.05 ($P < 0.05$).

3.4 Laboratory investigation of solutions/gels

This part of the investigation investigated two popular carbonated drinks, five energy gels and both a positive (Tropicana^{TM} smooth orange juice) and negative (Volvic^{TM} still mineral water) control. These are summarized in the table below (table 3.1).

Solutions Investigated	**Manufacturer**
Smooth orange juice (positive control)	Tropicana^{TM} (PepsiCo, Chicago, Illinois, United States)
Still mineral water (negative control)	Volvic^{TM} (Clairvic Spring, France)
Lucozade^{TM}	Ribena Suntory (UK)
Coca Cola^{TM}	Coca Cola^{TM} (Atlanta, Georgia, United States)
High 5 Orange energy gel^{TM}	High 5 (Brighton, UK)
High 5 Citric energy gel^{TM}	High 5 (Brighton, UK)
Isogel^{TM}	High 5 (Brighton, UK)
SiS Orange energy gel^{TM}	Science in Sport (London, UK)
SiS Blackcurrent energy gel^{TM}	Science in Sport (London, UK)

Table 3.1. The drinks and gels investigated for titratable acidity.

3.4.1 Titratable acidity

For each of the test solutions, five 20 ml samples were titrated to a pH of 7.0 using 0.1M sodium hydroxide whilst stirred continually with a magnetic stirrer set at a uniform rate. The pH electrode (Jenway 3510, Barloworld Scientific Ltd, Essex, UK) was calibrated at the start of each test using a standard purchased pre-made (Merck, Poole, UK) buffer solution of pH 7 (Figure 3.1).

Figure 3.1 Buffer solution, with magnetic stirrer and pH probe.

The initial pH and change in pH when test solutions were titrated with 0.1M of sodium hydroxide increments were recorded using a calibrated temperature compensated pH electrode (Jenway 3510, Barloworld Scientific Ltd, Essex, UK). The mean initial pH reading for each solution was recorded and the volume of 0.1M sodium hydroxide required to raise this to pH 7 was recorded. The mean and standard deviation of these values were calculated for all of the solutions/gels studied (figure 3.2).

Figure 3.2. Set up for titratable acidity laboratory work

To allow comparison of this study with others, the mean titratable acidity values were also expressed as the standardized titratable acidity (STA). This is defined as the mean volume of 0.1M sodium hydroxide needed to neutralize one litre of solution/gel. A formula for its calculation is found below.

STA= (mean volume in ml of 0.1M sodium hydroxide required to neutralise 20 ml of solution x 0.0001) x 50

For each drink/gel investigated, a total of 5 determinations of titratable acidity were made.

3.4.2 Contact Angle

The contact angle was measured to quantify the wettability of three different surfaces to seven solutions/gels. The three solid surfaces were, a glass side, a sectioned tooth and an ostrich egg. A single droplet of each test solution/gel was dropped from a reproducible distance (figure 3.3) from each sample surface.

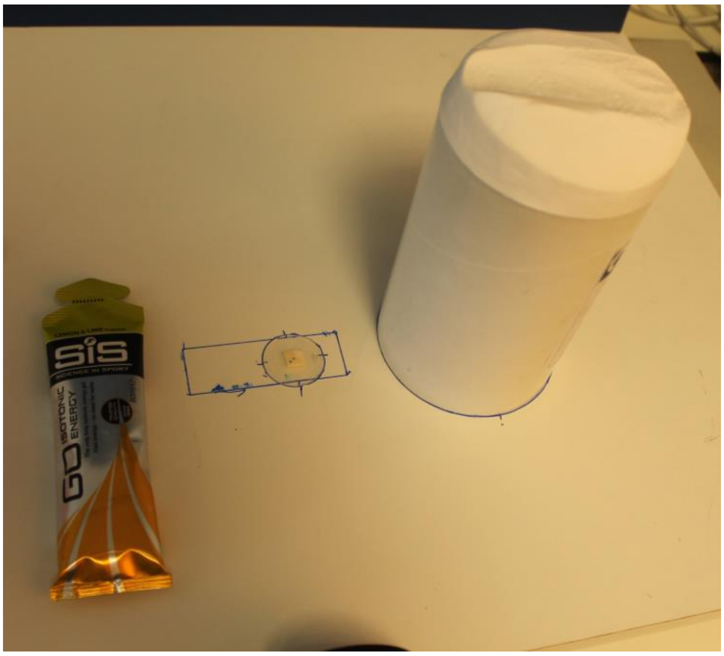

Figure 3.3. Postioning of solid surfaces and wrist rest from which droplet placed

A standardized wrist support was produced (figure 3.4) to ensure the drops were introduced to the surface from a reproducible point.

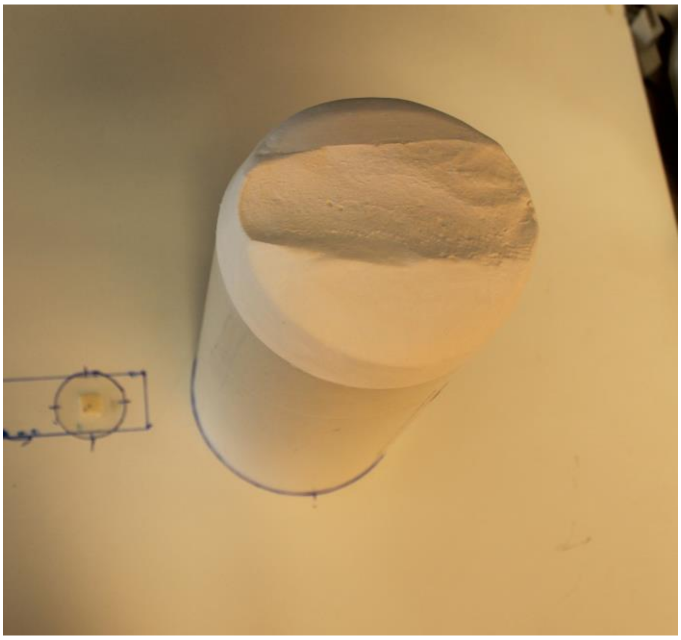

Figure 3.4. Custom made wrist rest from which droplets were placed

A camera tripod was set up with a Nikon camera and lens (figure 3.5) to view the drop upon the substrate from its side elevation of 0^0 to the substrate surface.

Figure 3.5. Camera set up to capture image of static contact angle

Once each drop was placed, a photograph was taken of the drop on the surface. This was repeated five times for each solution/gel tested on each of the three solid surfaces. The images produced were printed (size A4) and the contact angle measured by tracing on to tracing paper (Canson 90g/m^2 high transparency, France) the contact angles and measured with a protractor. An example of this can be seen in the images below (figures 3.6 and 3.7).   The mean contact angle for each solution/gel on the three different surfaces was calculated along with the standard deviation for each.

The resultant data was subjected to a two-way analysis of variance using the computer package Prism (Graph Pad Software Version 6, San Diego, California, USA) with post-hoc analysis to investigate any significant effect of substrate and drink/gel and their interaction.

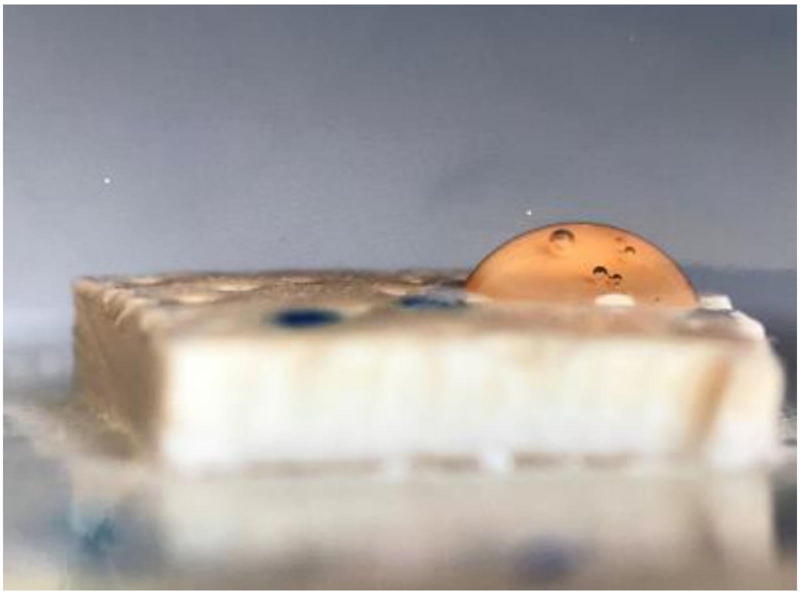

Figure 3.6. Drop of solution on Ostrich egg

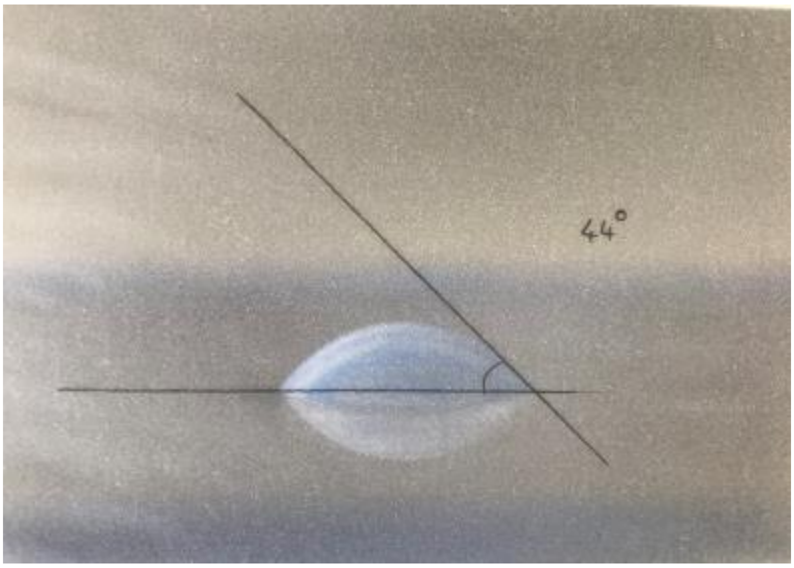

Figure 3.7. Contact angle traced and measured

3.4.3 Microhardness

The effect of a 20 minute exposure to each solution was investigated using specially prepared samples of extracted human enamel.

The teeth were collected and stored in Dundee Dental Hospital and School for research prior to September 2006. Chadwick & Blacker (2013) previously described the preparation of the teeth which is replicated as follows; teeth roots were removed and resultant crowns sectioned longitudinally to leave a 1 mm thick sagittal slice using a slow speed diamond saw (Isomet Buehler Ltd), at 450 RPM, under water coolant until the first signs of enamel were observed in the sections. Thereafter, 3 mm thick slices were prepared (Qutieshat *et al.* 2018). The fluoride history of these teeth was unknown. Following sectioning, each portion was temporarily glued to the base of a metset mounting mould (Buehler Coventry, UK) using a cyanoacrylate cement (Loctite® Precision Superglue, Henkel Consumer, Adhesives, Cheshire, CW7 3QY, UK) with buccal/palatal surfaces facing downwards. Afterwards, the mould was assembled and the tooth embedded in epoxy resin (Bonda Clear Casting Resin, Bondaglass Vost Ltd, Kent, UK) mixed according to the manufacturers instructions. Once set, the embedded portion of tooth in resin was removed from the mould and separated from the base mould. The buccal/ palatal enamel was finished flush with the surrounding mounting epoxy resin using a PM5 precision lapping and polishing machine (Logitech, Glasgow, Scotland) and a slurry of calcined Aluminium Oxide powder with a particle size of 9 μm (Logitech, Glasgow, Scotland) (figure 3.6).

Figure 3.8. Prepared tooth sample

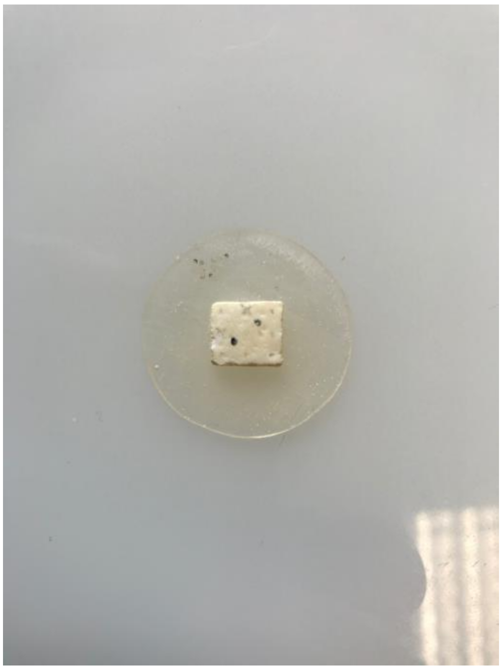

Figure 3.9. Prepared ostrich egg sample

Each specimen was numbered to allow for identification and allocated to a drinks treatment group. Each group consisted of five teeth and was allocated to one of the treatment gel groups as summarised in table 3.2.

Table 3.2. The gels investigated in the surface microhardness study.

Solutions Investigated	Manufacturer
SiS Apple energy gel	Science in Sport^{TM} (London, UK)
SiS Blackcurrant energy gel (B'currant)	Science in Sport^{TM} (London, UK)
SiS Citrus energy gel	Science in Sport^{TM} (London, UK)
SiS Pineapple energy gel (P'apple)	Science in Sport^{TM} (London, UK)
SiS Tropical energy gel	Science in Scport^{TM} (London, UK)

Prior to immersion in the test gels, the mean surface microhardness was tested. To achieve this, five separate baseline readings were recorded for each tooth sample. Each sample was then immersed in the gel it had been allocated to for 20 minute and each sample and the surface microhardness was then recorded five times for each sample.

Surface microhardness determination followed standard procedures used in the research laboratory where the work was carried out. These are contained in the Supervisors laboratory standards operating procedure (SOPS) file and reproduced here.

3.4.4 Surface microhardness measurement

Tooth surface hardness was used to assess the erosive potential of different flavours of the same brand energy gels tested. A TIV (Through Indenter

Viewing) (figure 3.8) hardness tester (GE Measurement & Control, Groby, UK) with a Vickers diamond (figure 3.9), under 9.8 N load was used to measure the surface hardness at 20 sites per specimen, 3 specimens were allocated to each gel.

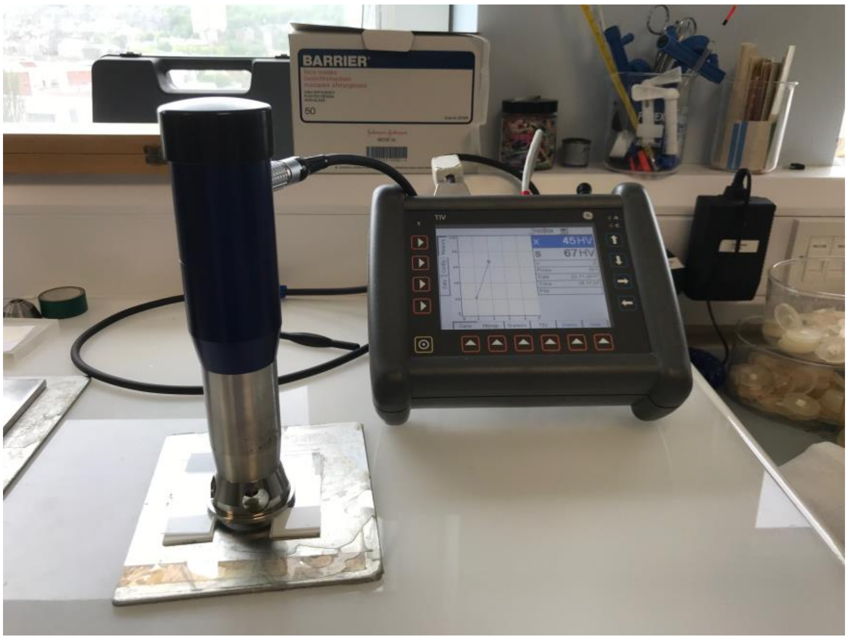

Figure 3.10. TIV Hardness tester

Figure 3.11. Vickers diamond contacting sample

4. RESULTS

4. Results

In this chapter, the results are presented under the same subheadings as those used in chapter 3- materials and methods.

Where statistical analyses were undertaken, the threshold for statistical significance is conventional. That is $P < 0.05$

4.1 Questionnaire to participants of amateur sport (Questionnaire 1)

This questionnaire was designed and administered to identify trends and levels of consumption of sports drinks and nutritional supplements. Once the questionnaire was constructed, the questionnaire was administered to a group of 10 participants to ensure it was easily understood and appropriate data could be extracted. 50 consenting individuals who competed in a range of amateur sports completed the questionnaire.

The questionnaire pilot upon 10 individuals demonstrated no need for modification. A repeat administration of this upon the same pilot sample gave an overall Kappa value of 0.87 (V. Good) with the Kappa values summaried in Table 4.1 for each component. The total number of finalised questionnaires returned was 50, all of which were suitable for analysis. Of the respondents, 30 were male and 20 female. In all the questionnaire results tables, which follow in this section, the number of respondents is given together with the percentage of the total sample that responded for that question. The raw data is used in all of the statistical comparisons made using the Chi square test. Percentages are included to facilitate the reader making normalized comparisons between the groups.

Question	Kappa	Strength
Gender	1	Perfect
Age	1	Perfect
History of wear	1	Perfect
History of Clenching	1	Perfect
Sport participated in	0.64	Good
Training Frequency	0.82	V. Good
Drinks consumed	1	Perfect
Frequency of drink consumption	0.70	Good
Gels consumed	1	Perfect
Frequency of gels consumption	0.74	Good
Supplements Consumed	1	Perfect
Frequency of supplement consumption	0.72	Good
Brands consumed	0.75	Good
Overall consumption of all products	0.82	V. Good
Method of consumption	0.91	V. Good

Table 4.1 Breakdown of Kappa value of the components of the pilot participant questionnaire

4.1.1 History of tooth wear

Table 4.2 demonstrates the number of male and female patients from the respondents who have been informed by a dentist they have tooth wear. A Chi square test revealed no statistical significant difference between males and females being informed of the presence of tooth wear by their dentist. (The Chi-square statistic is 1.55. The p-value is 0.21. This result is *not* significant at $p < 0.05$.)

	Male	Female
Informed of presence of tooth wear by dentist	5 (16.7%)	1 (5%)
Not informed of presence of tooth wear by dentist	25 (83.3%)	19 (95%)

Table 4.2. Subjects informed of presence of tooth wear

4.1.2 Consumption

Table 4.3 summaries the number of males and females who consume sports drinks along with the range and mean weekly portion consumption. A Chi square test revealed no statistical significant difference between males and females who consume sports drinks. (The chi-square statistic is 1.97. The *p*-value is 0.16. This result is *not* significant at $p < 0.05$.) However, there is a clear difference in the mean and range of portions consumed per week in males and females.

	Male	Female
Consume sports drinks (Percentage)	15 (50%)	6 (30%)
Don't consume sports drinks (Percentage)	15 (50%)	14 (70%)
Weekly portion consumption (Range)	11 (12-1)	2 (3-1)

Table 4.3. Frequency of sport drink consumption related to gender

4.1.2 Consumption

Table 4.4 summaries the number of males and females who consume energy gels along with the range and mean weekly portion consumption. A Chi square test revealed no statistical significant difference between males and females who consume energy gels. (The chi-square statistic is 2.04. The *p*-value is .015. This result is *not* significant at $p < 0.05$.) However, as with sports drink consumption, there is a trend clear difference in the mean and range of portions consumed per week in males and females.

	Male	Female
Consume energy gels (Percentage)	9 (30%)	10 (50%)
Don't consume energy gels (Percentage)	21 (70%)	10 (50%)
Weekly portion consumption (Range)	9 (10-1)	4 (5-1)

Table 4.4. Frequency of energy gel consumption related to gender

4.1.2 Consumption

Table 4.5 summaries the number of males and females who consume other sports supplements such as protein shakes/bars/lozenges along with the range and mean weekly portion consumption. A Chi square test revealed no statistical significant difference between males and females who consume other sports supplements.

(The chi-square statistic is 1.75. The *p*-value is 0.186. This result is *not* significant at $p < 0.05$.)

	Male	Female
Consume other sports supplements (Percentage)	13 (43.3%)	5 (25.0%)
Don’t consume other sports supplements (Percentage)	17 (56.7%)	15 (75.0%)
Weekly portion consumption (Range)	Max. 7 Min. 1 (6)	Max. 4 Min. 1 (3)

Table 4.5 Frequency of other sports supplement consumption related to gender

4.1.2 Consumption

The results of the survey were used to establish which drinks/gels were commonly used and a selection of these were chosen to tested further in the laboratory. The drinks/gels most commonly used by the participants of this survey are displayed in table 4.6.

Brands	Number	%
High5™	10	20
Lucozade™	9	18
SIS ™	6	12
Torq ™	4	8
USN Protein ™	3	6
Unknown	3	6
My protein ™	2	4
REGO ™	1	2
Reflex protein ™	1	2
NUUN ™	1	2
GU ™	1	2
Greenmonster ™	1	2

Table 4.6. The most common solutions/gels consumed by the participants.

The manufacturers of these drinks are summarized in table 4.7

Brands	**Manufacturer**
High5™	High5
Lucozade™	Lucozade Ribena Suntory Limited
SIS ™	SIS
Torq ™	Torq limited
USN Protein ™	Ultimate Sports Nutrition
Unknown	-
My protein ™	MYPROTEIN
REGO ™	SIS
Reflex protein ™	Reflex nutrition limited
NUUN ™	nuun
GU ™	GU Energy labs
Greenmonster ™	1

Table 4.7. The most common solutions/gels consumed by the participants and their manufacturer.

4.1.3 Sports recipients participated

The survey was distributed to a number of sporting groups and at a number of sporting events. As part of the survey we established which sports the participants of the survey commonly engaged in. These results are displayed in table 4.8.

Table 4.8. The sports recipients participated in.

Sport	Number	%
Running	31	62
Cycling	9	18
Football	5	10
Fitness	3	6
Weight training	3	6
Hillwalking	3	6
Swimming	2	4
Boxing	2	4
Circuits	1	2
Triathalon	1	2
Cricket	1	2
Rugby	1	2
Rowing	1	2

4.2 Questionnaire to General Dental Practitioners (Questionnaire 2)

This questionnaire was designed and administered to identify current practices and confidence levels in managing tooth wear of the local general dental practitioners. The questionnaire was circulated to local general practitioners registered to treat NHS patients in NHS Grampian (NHSG) trust and three reminder emails were sent via NHSG Practitioners Services. A total of 42 questionnaires were returned completed. Unfortunately, NHSG Practitioners services were unable to provide me with the number of dentist who were sent the questionnaire, meaning a return rate could not be calculated.

4.2.1 Years participants graduated

The total number of finalised questionnaires returned was 42, all of which were suitable for analysis. Respondents graduated from both the United Kingdom and abroad with graduation dates ranging from 1972 to 2018 (Table 4.9). In all the questionnaire results tables, which follow in this section, the number of respondents is given together with the percentage of the total sample that responded for that question. The raw data is used in all of the statistical comparisons made using the Chi square test. Percentages are included to facilitate the reader making normalized comparisons between the groups.

Graduated from dental school between and including these years	Number	%
1971-1980	1	2
1981-1990	7	17
1991-2000	7	17
2001-2010	12	29
2011-2018	15	36

Table 4.9. The year participants graduated from dental school.

4.2.2 UK versus International graduates

Table 4.10 demonstrates the number of UK gradates and International graduates who felt their undergraduate teaching in managing tooth wear prepared them for general practice. (The chi-square statistic is 1.02. The *p*-value is 0.31. This result is *not* significant at $p < 0.05$.)

	UK Graduates	International Graduates
Undergraduate degree prepared them for managing tooth wear (Percentage)	7 (21%)	3 (37.5)
Undergraduate degree didn't prepared them for managing tooth wear(Percentage)	27 (79%)	5 (62.5)

Table 4.10. Does country of education prepare dentists to manage tooth wear?

4.2.3 Pre 2000 graduates versus 2001 and onwards

Table 4.11 demonstrates the number of graduates from 2000 and earlier who feel their undergraduate teaching prepared them for managing tooth wear in practice compared to those who graduated from 2001 onwards (The chi-square statistic is 0.38. The *p*-value is 0.54). This result is *not* significant at $p < 0.05$. As well, this table also demonstrates the number who have attended postgraduate course on managing tooth wear (The chi-square statistic is 2.49. The *p*-value is 0.11. This result is *not* significant at $p < 0.05$).

Table 4.11. Experience of pre 2000 graduates compared to 2001 onwards

	Graduated in or before 2000	Graduated in 2001 or later
Undergraduate degree prepared them for managing tooth wear	4 (10%)	5 (12%)
Undergraduate degree didn't prepare them for managing tooth wear	11 (26%)	22 (52%)
Attended postgraduate course on tooth wear	11 (26%)	13 (31%)
Did not attend postgraduate course on tooth wear	4 (10%)	14 (33%)

4.2.4 Confidence in managing tooth wear

Table 4.12 demonstrates the level of confidence of the respondents in managing tooth wear on a scale of 1-5 along with the mean level of confidence being calculated as a confidence level of 2.71. The percentage of dentists who responded to each grade of confidence was also calculated. The level of confidence was demonstrated on the Questionnaire (appendix B), with 1 being not at all confident and 5 being very confident

Level of confidence	1	2	3	4	5	mean
Number	6	10	17	8	1	2.71
%	14	29	40	19	2	

Table 4.12. Confidence levels of dentists managing tooth wear

4.2.5 Specialist services and remuneration

In addition to the above, the following table (Table 4.13) demonstrated the number (percentage) of respondents who felt there was adequate access to specialist services to manage tooth wear cases and what was thought about possible remuneration if carried out in general practice. It was clear that neither access to specialist services and remuneration were perceived as adequate (2 respondents did not answer this question).

	Adequate	**Inadequate**
Access to specialist services	19 (45%)	23 (55%)
Remuneration (2 did not respond)	7 (17%)	33 (79%)

Table 4.13 Do dentists feel they have adequate access to specialist services and are they remunerated appropriately?

4.3 Titratable Acidity of Solutions

Five 20 ml samples of ten different shop bought sports energy gels or solutions where tested for their titratable acidity against 0.1M sodium hydroxide, the results were as follows.

4.3.1 Mean baseline pH reading, titratable acidity and standardized titratable acidity (STA)

The tables that follow give the mean baseline pH, titratable acidity and standardized titratable acidity (STA) for all of the solutions/gels studied including the controls together with the standard deviations of these observations.

Analysis of variance of the baseline pH readings and the titratable acidity of the solutions gels was carried out and revealed, for these parameters, highly significant difference ($P < 0.001$) between the drinks (table 4.14).

Table 4.14. ANOVA of pH readings

ANOVA	SS	DF	MS
Treatment (between columns)	9.98	7.00	1.43
Residual (within columns)	0.12	32.00	0.0037
Total	10.09	39.00	

From table 24 all solutions/gels studied with the exception of Volvic^{TM} still mineral water (negative control) had a mean baseline pH below the critical pH of 5.5.

Table 4.15. The mean baseline pH readings of test solutions/gels including positive and negative controls.

Solution/gel	Initial pH (mean)	Standard Deviation
Lucozade	3.91	0.02
High 5 Orange Gel	4.88	0.00
High 5 Citric Gel	4.70	0.01
Tropicana^{TM} smooth orange juice	5.00	0.07
Coca Cola^{TM}	3.79	0.06
High 5 Isogel	4.78	0.02
SIS orange Gel	5.13	0.08
SIS Blackcurrent Gel	5.16	0.11
Volvic^{TM} still mineral water	8.08	0.05

Table 4.16 displays the titratable acidity and from this it can be seen that Tropicana^{TM} orange juice (positive control) required the largest amount of 0.1M NaOH to bring its pH to 7.00 at 16.23 mls. This was followed by the High 5 isogel requiring 9.54 mls, the High 5 orange flavoured gel needed a mean of 9.4 mls, lucozade required 9 mls and the High 5 Citric flavoured gel 7.21 mls to reach a pH of 7.00. The remaining gels required a much smaller volume of 0.1M NaOH to reach our target 7.00 pH, with coke requiring 3.21 mls, SiS blackcurrent 2.35mls and finally SiS Orange 2.26 mls.

Table 4.16. The mean volume of 0.1M NaOH to obtain pH 7 reading of test solutions/gels including positive control.

Solution/gel	Mean volume (mls) 0.1M NaOH to achieve pH 7 (standard deviation)
Lucozade	9.01 (0.37)
High 5 Orange Gel	9.39 (0.17)
High 5 Citric Gel	7.21(0.13)
Tropicana^{TM} smooth orange juice	16.23 (0.28)
Coca Cola^{TM}	3.21 (0.31)
High 5 Isogel	9.54 (0.13)
SIS orange Gel	2.26 (0.04)
SiS Blackcurrent Gel	2.35 (0.13)

The mean values and standard deviation expressed in this table were the result of five experimental runs for each solution/gel.

A Tukey comparison of baseline pH means to localize the differences. There was no statistical significance (P >0.05) in mean baseline pH between; High 5 Orange gel and High 5 Isogel, High 5 Citric gel and High 5 Isogel, Tropicana^{TM} smooth orange juice and SIS Orange gel, Tropicana^{TM} smooth orange juice and SIS blackcurrant gel and SIS orange gel and SIS Blackcurrant gel. All other interdrink comparisons observed showed statistically significant differences (P <0.05) (Table 4.17)

Table 4.17. Results of Tukey Comparison of mean baseline pH measurements for drinks/gels investigated.

Versus	High 5 Orange gel	High 5 Citric gel	Tropicana^{TM} smooth orange juice	Coca Cola^{TM}	High 5 Isogel	SIS Orange Gels	SIS Blackcurrant Gels
Lucozade	****	****	****	*	****	****	****
High 5 Orange gel	–	**	**	****	NS	****	****
High 5 Citric gel	–	–	****	****	NS	****	****
Tropicana^{TM} smooth orange juice	–	–	–	****	****	NS	NS
Coca Cola^{TM}	–	–	–	–	****	****	****
High 5 Isogel	–	–	–	–	–	****	****
SIS Orange Gels	–	–	–	–	–	–	NS

NS = No statistical difference

* = P < 0.05

**= P < 0.01

***= P <0.001

****= P <0.0001

Table 4.18 give the mean titratable acidity of the drinks/gels tested and their standard deviation.

Table 4.18 The mean titratable acidity of solutions/gels tested.

Solution/gel	**Mean titratable acidity (mls of 0.1M NaOH) with SD**	**Standardised Titrateable Acidity (STA)**
Lucozade	9.01 (0.37)	4.50×10^{-2}
High 5 Orange Gel	9.29 (0.17)	4.69×10^{-2}
High 5 Citric Gel	7.21 (0.13)	3.60×10^{-2}
Tropicana™ smooth orange juice	16.23 (0.28)	8.12×10^{-2}
Coca Cola™	3.21 (0.31)	1.61×10^{-2}
High 5 Isogel	9.54 (0.13)	4.77×10^{-2}
SIS orange Gel	2.26 (0.04)	1.13×10^{-2}
SiS Blackcurrent Gel	2.35 (0.13)	1.12×10^{-2}

An analysis of variance of this data revealed highly significant (P <0.0001) differences between the drinks/gels (Table 4.19).

Table 4.19 ANOVA Titratable Acidity

ANOVA	SS	DF	MS
Treatment (between columns)	793.30	7.00	113.30
Residual (within columns)	1.57	32.00	0.049
Total	794.90	39.00	

A Tukey comparison of titratable acidity means to localize the differences. This is summarized in Table 4.20. which demonstrates there was no statistical significance (P >0.05) in mean titratable acidity between; Lucozade and High 5 Orange gel, High 5 orange gel and High 5 Isogel, SIS orange gel and SIS Blackcurrant gel only. All other interdrink comparisons observed showed statistically significant differences (P <0.05).

Table. 4.20 Results of Tukey Comparison of mean titratable acidity measurements for drinks/gels investigated.

Versus	High 5 Orange gel	High 5 Citric gel	Tropicana™ smooth orange juice	Coca Cola™	High 5 Isogel	SIS Orange Gels	SIS Blackcurrant Gels
Lucozade	NS	****	****	****	*	****	****
High 5 Orange gel	–	****	****	****	NS	****	****
High 5 Citric gel	–	–	****	****	****	****	****
Tropicana™ smooth orange juice	–	–	–	****	****	****	****
Coca Cola™	–	–	–	–	****	****	****
High 5 Isogel	–	–	–	–	–	****	****
SIS Orange Gels	–	–	–	–	–	–	NS

NS = No statistical difference

* = P < 0.05

**= P < 0.01

***= P <0.001

****= P <0.0001

4.4 Contact Angle.

The tables below (table 4.21) demonstrates the mean contact angles with standard deviations for Lucozade, High 5 Orange gels, Orange juice, Coca Cola, High 5 Isogel, SIS Orange Gels and Volvic mineral water on a tooth, Ostrich egg and a glass slide. The results demonstrate that regardless of the liquid, the glass slide surface always produced the lowest contact angle and therefore the solid surface with the greatest wettability of the 3 tested. On ostrich egg and tooth surface, water had the greatest contact angle and therefore the lowest wettability of all liquids/solutions tested.

	Ostrich egg (Mean)	Ostrich egg (SD)	Tooth (Mean)	Tooth (SD)	Glass slide (Mean)	Glass slide (SD)
Lucozade	55.83	6.55	43.17	5.57	49.83	3.66
High 5 Orange gel	46.00	5.48	36.17	7.33	32.50	1.76
Tropicana™ smooth orange juice	48.83	9.33	54.50	2.67	45.00	2.45
Coca Cola™	54.50	3.99	34.33	2.07	30.67	3.83
High 5 Isogel	48.83	7.14	25.00	5.66	20.83	1.72
SIS Orange Gels	64.50	3.89	54.50	2.67	45.00	2.45
Volvic™ still mineral water	70.67	4.18	59.50	5.24	27.83	3.37

Table. 4.21 Mean contact angles (degrees) of test gels/solutions and their standard deviations.

4.5 Contact angle and STA combined.

The table below (table 4.22) demonstrates the mean contact angle of each solution on the tooth surface and the STA of each of these solutions.

	STA	Mean contact angle on tooth
Lucozade	4.50×10^{-2}	43.17
High 5 Orange gel	4.69×10^{-2}	36.17
Tropicana™ smooth orange juice	8.12×10^{-2}	54.5
Coca Cola™	1.61×10^{-2}	34.33
High 5 Isogel	4.77×10^{-2}	25
SIS Orange Gels	1.13×10^{-2}	54.5

Table 4.22. Mean contact angle and STA of test solutions/gels

A two-way analysis of the contact angle measurements revealed significant effects ($P < 0.0001$) of drink/gels, substrate (glass slide, ostrich egg and tooth) and their interaction. The results of localization of these differences according to substrate type are summaries in table's 4.23- 4.25. From these, it is clear that;

4.5.1 Ostrich egg

There was statistical significant difference ($P < 0.05$) in contact angle on the ostrich egg substrate between Lucozade and Volvic^{TM} Mineral water, High 5 Orange gel and Volvic^{TM} Mineral water, High 5 Orange gel and SIS orange gel, Tropicana^{TM} smooth orange juice and SIS Orange gel, Tropicana^{TM} smooth orange juice and Volvic^{TM} Mineral water, Coca Cola^{TM} and Volvic^{TM} Mineral water, High 5 Isogel and SIS Orange Gels, High 5 Isogel and Volvic^{TM} Mineral water. All other interdrink comparisons observed showed no statistically significant difference ($P > 0.05$).

Table 4.23. Results of Tukey Comparison of mean contact angle measurements for drinks/gels investigated on Ostrich egg substrate

Versus	High 5 Orange gel	Tropicana™ smooth orange juice	Coca Cola™	High 5 Isogel	SIS Orange Gels	Volvic™ Mineral water
Lucozade	NS	NS	NS	NS	NS	***
High 5 Orange gel	–	NS	NS	NS	****	****
Tropicana™ smooth orange juice	–	–	NS	NS	***	****
Coca Cola™	–	–	–	NS	NS	***
High 5 Isogel	–	–	–	–	***	****
SIS Orange Gels	–	–	–	–	–	NS

NS = No statistical difference

* = P < 0.05

**= P < 0.01

***= P <0.001

****= P <0.0001

4.5.2 Tooth

There was no statistical significance ($P > 0.05$) in contact angle on the tooth substrate between; Lucozade and High 5 Orange gel, Lucozade and Coca Cola^{TM}, High 5 Orange gel and Coca Cola^{TM}, Tropicana^{TM} smooth orange juice and SIS Orange Gel, Tropicana^{TM} smooth orange juice and Volvic^{TM} Mineral water, Coca Cola^{TM} and High 5 Isogel and SIS orange gel and Volvic^{TM} Mineral water. All other interdrink comparisons observed showed statistically significant difference ($P < 0.05$).

Table 4.24. Results of Tukey Comparison of mean contact angle measurements for drinks/gels investigated on tooth substrate

Versus	High 5 Orange gel	Tropicana™ smooth orange juice	Coca Cola™	High 5 Isogel	SIS Orange Gels	Volvic™ Mineral water
Lucozade	NS	*	NS	****	*	***
High 5 Orange gel	–	****	NS	*	****	****
Tropicana™ smooth orange juice	–	–	****	****	NS	NS
Coca Cola™	–	–	–	NS	****	****
High 5 Isogel	–	–	–	–	****	****
SIS Orange Gels	–	–	–	–	–	NS

NS = No statistical difference

* = P < 0.05

**= P < 0.01

***= P <0.001

****= P <0.0001

4.5.3 Glass Slide

There was no statistical significance (P >0.05) in contact angle on the glass slide between; Lucozade and Tropicana^{TM} smooth orange juice, Lucozade and SIS Orange gels, High 5 Orange gel and Coca Cola^{TM}, High 5 Orange gel Volvic^{TM} Mineral water, Tropicana^{TM} smooth orange juice and SIS Orange Gel, Coca Cola^{TM} and High 5 Isogel, Coca Cola^{TM} and Volvic^{TM} Mineral water and SIS orange gel and Volvic^{TM} Mineral water. All other interdrink comparisons observed showed statistically significant difference (P <0.05).

Table 4.25. Results of Tukey Comparison of mean contact angle measurements for drinks/gels investigated on glass slide.

Versus	High 5 Orange gel	Tropicana^{TM} smooth orange juice	Coca Cola^{TM}	High 5 Isogel	SIS Orange Gels	Volvic^{TM} Mineral water
Lucozade	****	NS	****	****	NS	****
High 5 Orange gel	–	*	NS	*	*	NS
Tropicana^{TM} smooth orange juice	–	–	**	****	NS	****
Coca Cola^{TM}	–	–	–	NS	**	NS
High 5 Isogel	–	–	–	–	****	****
SIS Orange Gels	–	–	–	–	–	NS

NS = No statistical difference

* = P < 0.05

**= P < 0.01

***= P <0.001

****= P <0.0001

4.6 Surface microhardness

Microhardness testing of human dental hard tissue was carried out following exposure to 5 different flavours of the same brand of energy gel to investigate if flavour would impact on surface microhardness.

The effect of different flavoured energy gels on Surface Mircohardness

Table 4.26 summarises the pre and post application mean hardness values, with their standard deviations, for the gels. D'Agostino and Pearson normality test demonstrated that this data was not normally distributed. A Kruskal-Wallis test revealed highly statistically significant difference ($P<0.0001$) between these values. Localisation using Dunn's multiple comparisons test demonstrated significant deteriorations in mean hardness where the following gels had been applied.

- Blackcurrant $P < 0.01$
- Tropical $P < 0.01$

	AP	APP	BC	BCP	CS	CSP	PA	PAP	TL	TLP
Mean Vickers Hardness	67.50	63.45	65.23	58.20	68.95	63.75	56.77	55.57	56.82	43.57
Standard deviation	4.88	4.86	9.24	6.77	9.40	11.28	20.95	14.33	12.61	11.76

Table 4.26. Mean microhardness results pre and post exposure.

AP- Apple pre exposure

BC- Blackcurrant pre exposure

CS- Citrus pre exposure

PA- Pineapple pre exposure

TL- Tropical pre exposure

APP- Apple post exposure

BCP- Blackcurrant post exposure

CSP- Citrus post exposure

PAP- Pineapple post exposure

TLP- Tropical post exposure

Table 4.27. Variations in the hardness values of the various experimental groups.

Versus	AP	BC	CS	PA	TL	APP	BCP	CSP	PAP	TLP
AP	–	NS	NS	NS	***	NS	****	NS	****	****
BC	–	–	NS	NS	NS	NS	**	NS	NS	****
CS	–	–	–	NS	****	NS	****	NS	****	****
PA	–	–	–	–	NS	NS	NS	NS	NS	****
TL	–	–	–	–	–	NS	NS	NS	NS	***
APP	–	–	–	–	–	–	NS	NS	NS	****
BCP	–	–	–	–	–	–	–	*	NS	**
CSP	–	–	–	–	–	–	–	–	NS	****
PAP	–	–	–	–	–	–	–	–	–	***

AP- Apple pre exposure

BC- Blackcurrant pre exposure

CS- Citrus pre exposure

PA- Pineapple pre exposure

TL- Tropical pre exposure

APP- Apple post exposure

BCP- Blackcurrant post exposure

CSP- Citrus post exposure

PAP- Pineapple post exposure

TLP- Tropical post exposure

NS = No statistical difference

* = P < 0.05

**= P < 0.01

***= P <0.001

****= P <0.0001

On comparing variation amongst the hardness values at baseline and following gel application using a Kruskal-Wallis test with Dunn's multiple comparison, there were highly statistically significant differences ($P < 0.01$) in all cases except;

- Pre Apple Specimen 2 versus Pre Apple Specimen 3,
- Pre Blackcurrant Specimen 1 versus Pre Blackcurrant Specimen 2 and
- Post Tropical Specimen 1 versus Post Tropical Specimen 2,

where no statistical differences were found.

There can be a number of reasons to account for no statistical differences in the specimens above names;

1. Variation in the area tested, it can be difficult to test the exact same surface area and following exposure, should the area tested post exposure still have some residual gel or be wet following washing of the surface, an erroneous reading may be obtained.
2. Difference in thickness of the tested area may have an impact of the original and post exposure surface microhardness
3. The presence of enamel and dentine pre and post exposure. The area tested on each tooth sample is assumed to have the same structure however, there maybe variation in dentine/enamel presence and structure between the pre and post area tested.

4.7. Principal findings

- There would appear to be no statistically significant difference in the consumption habits of males and females in relation to sports drinks/gels/supplements.

- There would appear to be no statistically significant differences associated to year of graduation or country of graduation when comparing confidence of general dentists managing tooth wear.

- There is a feeling current remuneration is inadequate for managing tooth wear on general practice.

- Standard titratable acidity of solutions/gels can vary but they have been shown to be a potential cause of dental erosion.

- Ostrich egg is not a suitable substitute for tooth *in vitro* with regards to wettability

- Microhardness of tooth surfaces maybe effected by energy gels and therefore potentially contribute to dental erosion. Also, the flavour of the gel may have an impact.

5. Discussion

5. Discussion

5.1 Introduction

Overall, this study aimed to identify the erosion potential of sports drinks/gels. The secondary aims were to explore common trends and knowledge of those who consumed these drinks in relation to dental health. Finally, I also hoped to identify how general dental practitioners, served by the restorative service of my dental hospital, felt regarding management of tooth wear in general practice.

5.2 Participant Questionnaire (Questionnaire 1)

When discussing the findings of any questionnaire it is important to first discuss the return rate so that any findings may be contextualised. In this study, I handed out the first questionnaire and all 50 questionnaires were completed could be included in the study giving a 100% return rate. This return rate is likely related to my presence in handing out and collecting the completed questionnaires on the same day. Had I not handed out and waited for these to be completed I suspect the return rate would not have been as high. That being said it has to be noted that 50 is a small sample size to make definitive conclusions regarding the general population but this questionnaire did allow me to identify trends and brands for laboratory testing. It is however stated in the literature that there is no agreed standard for an acceptable minimum response other than a 75% minimum is consider good (Bharm *et al*, 2005)

Currently, there is no data available on the habits and trends of non-elite athletes and their consumption of sport energy gels. This study revealed that females more frequently consumed over the counter carbohydrate energy gels than males (50% compared to 30% (table 4.4)). Males consumed almost double the mean portion of gels compared to females of 3.06 compared to 1.8. However, when looking at sport drink consumption, a higher percentage of females consumed these than their male counterparts (females 30% compared to male 50% table 4.3). Again however, males mean portion consumption was significantly higher than females, 2.88 and 1.7 respectively.

It is interesting to note that a high proportion of both males and females who took part in this study frequently take sports supplements. Hansen (Hansen *et al.* 2013) demonstrated the additional benefit to endurance athletes in consuming energy gel supplements during a marathon although these benefits were 5% less than those derived from a scientifically based nutritional strategy. A scientifically based nutritional strategy can be expensive and is usually reserved for elite athletes. However, the continuing accessibility of sports gels and supplements mean amateur athletes can enhance performance and recovery by regularly consuming these gels. This may account for their increase in popularity. The psychology of drink selection depends on a number of factors such as cost, taste, availability, promotional offers, health qualities, peer selection and advertising. Although not explored in this survey, these factors may account for the observed behaviour differences between males and females in this respect (Kassem *et al.* 2003 & Naska *et al*. 2010).

As this questionnaire was completed by people who participated in amateur sport, it must be recognised that the majority were actively involved in a high level of sporting activity which may therefore lead to selection bias. Despite the rise in sales of these drinks/supplements, by targeting a population who are more likely to use these, we have to be mindful of how this information can be extrapolated to the general population. Perhaps, a better way to conduct this questionnaire would be to obtain a larger sample size but not target those who just participate in sport, this may have reduced the potential for selection bias and potentially shown some other interesting results such as; consumption of energy drinks amongst under 18's, consumption of energy drinks/carbonated drinks in combination with alcohol or even the use of dietary supplements taken by those dieting or enhancing their calorific intake for medical reasons. These were however not the aims of the present work and the questionnaire returns served to identify gels for further laboratory study in this work.

5.3 Dentist Questionnaire (Questionnaire 2)

The second questionnaire was distributed via the practitioner services and was sent on three separate occasions to an unknown number of general dental practitioners working in the NHS Grampian health board. Unfortunately, despite three reminder emails sent on a weekly basis and allowing for a week between each email, only 42 completed questionnaires were returned. As mentioned previously, unfortunately practitioner's services were unable to provide me with the number of dentists this questionnaire was sent to and as such a return rate could not be calculated. Despite not knowing the exact figure it is estimated almost 350 dentists would have received this questionnaire, which makes a return of 42 very disappointing. The potential response rate to survey based research has improved over the years according to Fincham (2008) and so it is disappointing that a representative sample could not be obtained for this survey. With such a small percentage return rate there is a significant non-response bias and also selection bias as those who returned the questionnaire could potentially be replying to express a more negative or positive view and therefore not representative of the population of general dental practitioners in NHS Grampian. Cook *et al*. (2000) pointed out that "response representativeness is more important than response rate in survey research. However, response rate is important if it bears on representativeness" and unfortunately in this case I believe it does. With hindsight, it may have been beneficial to incentivise returns as suggested by others (Bharm *et al.* 2005). However, I felt that an opportunity to express opinion regarding the services at their disposure would have been incentive enough. It was also disappointing that a management system could not track effectively to whom questionnaires were sent. This has implications for using this method for future research and also for their required record keeping of their performance.

5.4 Laboratory study

In this work, three laboratory tests were carried out to investigate the potential of test gels/solutions to bring about dental erosion *in-vitro*- acid/base titration, contact angle measurements and Vicker's surface microhardness testing.

As is the case with all laboratory studies, there are limitations in replicating the conditions of the oral environment, which in itself may have an impact on the development and progression of tooth wear. This is noted by Rees (2004) who explained tooth surface loss may be up to 10 fold greater under *in-vitro* conditions compared to intra-orally, due to the more severe conditions created within the laboratory. This being said, there are a number of advantages in carrying out *in-vitro* testing, namely; one variable at a time can be studied in standardised conditions, multiple tests can be carried out in a shorter period of time and importantly there is no risk to human subjects.

5.4.1 Titratable acidity

The method used to measure baseline and titratable acidity were similar to that used in previous reported studies (Blacker & Chadwick 2013). The volume of drink volume of gel used was 20 mls. This was selected as sports gels come usually in small pouches of between 40-60 mls. Although, these gels are viscous and previous studies have used larger samples of 100 mls (Blacker & Chadwick 2013) when testing thicker substances, it is not necessary to test more than would be consumed at any one time. In addition 20 mls has been used in other studies as an appropriate test volume (Rees *et al*. 2006).

The stirring method used employed a non-heating magnetic stirrer, set at the highest stirring rate, this was selected to account for the high viscosities of the gels tested. This was kept constant throughout the testing despite a range of viscosities of test solutions since it has been previously demonstrated by Shellis et al 2005, that stirring rate can have an effect of dissolution (Shellis *et al.* 2005).

The number of mls of 0.1M NaOH required to bring about neutrality of the drinks was reported; the standardised titratable acidity (STA) is also given as recommended by Syed and Chadwick (2009) to allow for inter-study comparisons. No previous literature was however available examining the pH or titratable acidity of sports gels. There were significant differences between the test solutions in terms of both baseline pH and titratable acidity. It was interesting to note that all test solutions with the exclusion of the negative control had a baseline pH of less than 5.2 (table 4.14) and that there was variation in STA between brands and flavours of energy gel, for example High 5 Orange flavoured gels required a mean of 9.4 ml of 0.1M NaOH compared to 7.21 ml for the citric flavoured version of the same gel, whilst SIS orange and Blackcurrent gels only required 2.26 ml and 2.35 ml respectively. These variations are likely due to complex interplay of different acid constituents and worthy of future chemical analysis.

There are a number of potential sources of error when carrying out titratable acidity testing;

1- **Temperature.** The bench experiment to determine titratable acidity was carried out over a number of days. The temperature of the lab and the solutions were not standardised and this could have an impact on the pH of a solution.
2- **Human error.** As with all bench experiments there is a possibility for human error, in this case that may be the reading from the pipette of the number of mls of 0.1M NaOH required to achieve a pH of 7. Another potential human error is control of the drop rate of 0.1M NaOH so that one final drop altered the pH to 7. If this drop rate was not controlled well, the number of mls required could be overestimated.
3- **Time.** As the pH of each sample increased, the drop rate of NaOH was reduced so that one final drop achieved a pH of 7. With the stirrer in place, a standardised drop rate could have been used affording time for the pH meter to register a change in pH.

4- **Solutions tested.** There was not consistency throughout all experiments with the solutions/gel used. This, to some degree prevents comparing how each solution/gel figured across multiple tests. In hindsight, it would have been advantageous to use the same solutions/gels in all experiments. However, I did not do this as in the titratable acidity experiment my aim was to explore the STA of commonly used products whilst in the contact angle experiment; the aim was to test the wettability of the solutions/gels. As the gels regardless of flavour had the same viscosity I felt they would provide the very similar results regardless of flavour. Finally, with regards to hardness testing, I aimed to test how flavours of the same product may impact dental hard tissues as such; the same brand of gel was used with the only variation of the gel being the flavour.

5.4.2 Contact Angle

The measurement of contact angles appears to be simple and straightforward. Unfortunately, this impression is very deceptive, and leads to many misunderstandings. From a technical point of view, the sessile drop method is frequently used, since this is the most convenient method (Marmur 2006). This however comes with a number of disadvantages; it only allows assessment on a macroscopic way when the surface of the substrate tested for wettability is indeed rough on a microscope level. Also, it assumes that the solution is not dynamic when in fact it maybe receding.

During this experiment, the sessile drop method was used and repeated six times on each surface for each test solution to establish a mean. The drops were ideally placed in the same position and dropped onto the substrate from the same height since this would impact on the spread of the solution. This is a commonly used technique due to its simplicity but also allows potential for testing errors to occur.

It may at first glance seem strange to examine wetting, by measurement of contact angle, upon ostrich eggshell. In view of the increasing difficulty of acquiring human tooth samples for dental research (Qutieshat, 2015) the laboratory where the present work was undertaken was exploring alternative substrates. Such eggshell has some similarities to human tooth structure being a natural biocomposite comprising an organic and mineral matrix of which 97.4 % is calcium carbonate (Yadao *et al*, 2004) arranged in a vertical crystal layer that is amorphous and has no evidence of porosities (Cooper *et* *al*, 2009). It has been shown to give similar results to human tooth substance when exposed to drinks of high erosive potential in an artificial mouth designed to evaluate erosiveness using surface profile, micro-hardness and mineral elution measures (Qutieshat, 2015). It therefore had pedigree for investigation in this work despite the unfavourable outcome as regards contact determination equivalence compared to human tooth substance.

Table 4.19 demonstrates significant differences between the contact angles achieved on glass, tooth and ostrich egg when tested with the same solutions. This would suggest that ostrich egg and glass would not be an appropriate experiment substitute for tooth structure when testing contact angle in the future. Similarly, a glass slide would not be an appropriate tooth substitute for further studies. Given the difficulty in accessing human teeth for such research an appropriate substitute should be sourced for future research purposes.

There are a number of potential sources of error when carrying out contact angle determination;

1- **Reproducibility.** As standardised set up was used for each photograph to be taken however, each solution/gel was dropped from a height on to the test surface. Despite a customised arm rest being used there is always potential for slight difference in the height from which the drop was made, this would impact the relationship between solution and surface.

2- **Recording results.** Results were measured by calculation of the angle by tracing on to tracing paper; there is always the potential for human error in this process.

3- **Solutions tested.** As previously mentioned there was inconsistency in the solutions/gels tested in the different lab tests preventing cross-examination of results. For example, Blackcurrant SIS gels were used in the microhardness experiment and the STA experiment but their contact angle was not investigated, since it was felt the viscosity of all gels would lead to similar contact angle results. In hindsight, it would be beneficial to be consistent with all substances to allow comparison of results across all experiments.

4- **Specimen.** Although the experiment was run in a controlled fashion it can be argued that the extent of the wettability may be affected by structural variations of the specimens.

5.4.3 Surface Microhardness

This part of the study aimed to assess the effects of immersion of prepared human tooth tissue in different flavours of sports gels upon the surface hardness. Due to the nature in which the teeth were prepared it was inevitable that some of the test areas would have included subsurface enamel or dentine, which may have resulted in, elevated measurements, as dentine is softer than enamel and more susceptible when exposed to acidic solutions.

In this study, a TIV (Through Indenter Viewing) hardness tester (GE Measurement & Control, Groby, UK) with Vickers diamond, under a 9.8 N load, was used to measure the surface hardness. Its application was far less fatiguing than using a conventional Vickers hardness microscope. Its use in dental research so far is limited with only one published study to date using a TIV in dental research (Quiteshat *et al.* 2018). Some have suggested conventional Vickers hardness testers may demonstrate a lack of sensitivity for their deeper depth penetration abilities may be hindered by contact of the indenter with the underlying sound enamel.

From the results, it is evident that all flavours of gel reduced the surface microhardness however tropical had significantly reduced this more compared to the others. As I only tested one brand of gel in this part of the study, I did not test the High 5 gels which has a significantly higher STA which may have demonstrated more of an impact on the surface microhardness compared to the SIS gels which actually had a low STA. Also, the STA of the tropical gel was not investigated and this gel had the biggest impact on surface microhardness.

That being said, Blackcurrant SIS gels were tested for both STA and microhardness and demonstrated to have a statistically significant effect on both accounts.

Thus demonstrating that flavour is a significant factor in the effect gels has on the potential for erosion of human dental hard tissue. It has been demonstrated in other work however not specifically in relation to energy gels but with herbal tea, that flavouring may impact erosion potential.

There are a number of potential sources of error when carrying out titratable acidity testing;

1- **Solutions tested.** As previously mentioned there was inconsistency in the solutions/gels tested in the different lab tests preventing cross-examination of results. For example, only SIS gels were used in the microhardness experiment whilst there was a range of brands used for the STA experiment.
2- **Specimen.** Although the experiment was run in a controlled fashion it can be argued that the extent of the wettability may be affected by structural variations of the specimens.

5.5 Conclusions

It has been demonstrated in the literature how significant an issue tooth wear has become and its prevalence continues to increase. That being said, we must ensure patients are appropriately educated on prevention and general dental practitioners have appropriate training, knowledge, access to specialist services and remuneration to manage these patients.

There would appear to be significant differences in the composition of different sports gels and demonstrated previously by Zhang *et al* 2015. This study also demonstrates this particularly in relation to the complexity of the acid chemistry not only between differing brands and also different flavours.

Bashir *et al.* (1995) found that clearance of citric acid from the oral environment by saliva following a citric acid rinse was rather quick, with 90% of acid eliminated after the first minute although clearance patterns vary from individual to individual. The concern with sports gels is their use is concentrated during a period when saliva flow and consistency has been shown to be altered (Ligtenberg at al. 2016).   With this in mind although not demonstrated to be highly erosive, the time in which they are used may increase the risk of developing dental erosion.

5.5 Conclusion

Within the limitations of this study:-

1. There is need for better education concerning the risks related to the dentition posed by the consumption of sports gels/solutions.
2. All solutions tested had a low baseline pH with great variation in titratable acidity demonstrating the variable risk of dental erosion depending on the brand and flavour consumed.
3. Ostrich eggs and glass slides were found to be unsuitable replacements for tooth tissue when testing contact angle of the gels.
4. All flavours of sports gels tested for their effect on surface microhardness produced negligible changes with the exception of the tropical and blackcurrant flavoured gels.

5.6 Future work

This study has demonstrated a number of issues, which could be further investigated.

- I believe the questionnaire to dentists provided a glimpse to a wider problem. This posed a number of questions; Are undergraduates receiving appropriate training to confidently manage mild to moderate tooth wear in practice, is there still a place for tooth wear in the NHS specialist services given the current pressures, is there scope to develop a management strategy starting with education of the general public regarding the contribution diet has on tooth wear and how economical is managing such cases?

- Given the challenges of obtaining human teeth for dental research, what suitable, sustainable and readily available substitutes are out there?

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7. Appendices

Appendix A

UNIVERSITY OF DUNDEE

Dental School

UNIT OF RESTORATIVE DENTAL CARE
Sections of Operative Dentistry, Fixed Prosthodontics and Endodontology
Section of Integrated Oral Care

Toothwear Questionnaire

1. You

Male/Female (Please circle)

What age are you? Years

Have you been aware/or your dentist informed you that your teeth are wearing/becoming shorter or more transparent? Yes/No

Are you aware of grinding or clenching your teeth? Yes/No

Which sport do you mainly participate in?
……………………………………………………………………. (Please specify)

How often do you train? ____

2. Dietary

Do you ever consume sports drinks? Yes /No

If yes, how many per week? ____

Do you ever consume energy gels? Yes/No

If yes, how many per week? ____

Do you ever consume sports supplements (protein shakes/creatine)? Yes/No

If yes, how many per week? ____

3. Consumption

Which sport drinks, energy gels or protein supplements do you most commonly use?
____ (Please specify)

How often do you consume this? ____

How do you drink this (using a straw/from a glass/sports bottle/consumed in one go or over a longer time period)? ____

Thank you for taking time to complete the questionnaire.
Should you have any queries please contact W. Keys
William.keys@nhs.net

Appendix B

DUNDEE

Dental School

UNIT OF RESTORATIVE DENTAL CARE
Sections of Operative Dentistry, Fixed Prosthodontics and Endodontology
Section of Integrated Oral Care

Toothwear Questionnaire

1. What year did you qualify as a dentist? ______________________

2. Which Dental School did you qualify from? ______________________

3. Do you feel your undergraduate studies prepared you for managing toothwear in general practice?

   Yes/No (please delete as appropriate)

4. If No, why not?
   ____
   ____
   ____

5. Have you been on any postgraduate toothwear courses?

   Yes/No (please delete as appropriate)

6. Do you feel you have easy access to specialist care/advice on managing toothwear?

   Yes/No (please delete as appropriate)

7. In the last 12 months how often have you sought specialist opinion for a toothwear case?

   Never ☐ 1-5 ☐ 6-10 ☐ 10+ ☐

8. How confident (scale 1-5) are you in managing toothwear in general dental practice? (please circle)

Not confident at all		somewhat confident		Very confident
1	2	3	4	5

9. Do you feel under the current fee structure toothwear is appropriately remunerated.

   Yes/No (please delete as appropriate)

Thank you for taking time to complete the questionnaire.
Should you have any queries please contact W. Keys
William.keys@nhs.net

Appendix C

University of Dundee

University of Dundee Schools of Nursing & Health Sciences and Dentistry Research Ethics Committee (SREC)

University of Dundee
Dundee
DD1 4HJ

24 January 2019

Dear Will,

Application Number: UoD\SDEN\2019001_Keys

Title: An investigation of into Scottish general dental practitioners view on training, management and support with regards patients suffering non-carious tooth surface loss; a questionnaire

I am writing to advise you that your ethics application has been reviewed and approved independently by reviewers on behalf of the SREC.

If your project data can be linked to an identifiable individual, you must notify the University Data Protection Officer, Mr Alan Bell a.z.bell@dundee.ac.uk.

Approval is valid for three years from the date of this letter. Should your study continue beyond this point, please request a renewal of the approval.

Any changes to the approved documentation (e.g., study protocol, information sheet, consent form) must be approved by this SREC.

Yours sincerely

Professor Mark Hector
Head of Department and Dean of Dentistry
On behalf of Schools of Nursing & Health Sciences and Dentistry Research Ethics Committee